Edited by
Prof. Dr. Kemal ÇELİK

Medicinal Beekeeping for BeeKeepers

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Medicinal Beekeeping for BeeKeepers
(MEDI-BEEB)
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Bee Products for Traditional and Complementary Medicine: Collection, Storage, Processing

Edited by Prof. Dr. Kemal ÇELİK

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Editor : Prof. Dr. Kemal ÇELİK
Writer : Assoc. Prof. Dr. Anželika Dautartė, Assoc. Prof. Dr. Barbara Król, Prof. Dr. Harun BAYTEKIN, Dr. Andrea Palomba, Dr. Kemal ÇELİK, Dr. Maja Słupczyńska, Dr. Massimo Canalicchio, Prof. Dr. Murat YILMAZ, Selda MANAV, Turgut KÜÇÜK, Alkan ÇAĞLI, Bircan AKPINAR
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T / (312) 341 36 67 - GSM / (533) 093 78 64
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soncagyayincilik@gmail.com
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uzun@uzundijital.com

PREFACE

Bees are among the most industrious and hardworking creatures in the world. Despite their tiny wings, these creatures having large bodies are energetic enough to flap their wings 11,400 times per minute to carry their bodies. According to scientists, the bees were on the earth even when we were not. While the first known bee fossil in the world was about 100 million years old, the first fossil belonging to us, humans, was about 300 thousand years old. These creatures have 6 legs, 5 eyes and 2 pairs of wings and 170 scent receptors who say "hello" to 2 million flowers for a pound of honey. All the things that these living creatures collect from nature and process are used in health protection and treatment. The first information about the use of bee products in human health is very old, dating back to ancient times, and the oldest findings were unearthed in the Catalhoyuk excavations in Anatolia (7000 BC). On the other hand, even though we rank in the top place among our rivals in terms of our hive presence and honey production, we are not yet at the targeted level regarding the awareness, the production, and the use of bee products. Despite its rich potential, Anatolia, which is the hearth of many civilizations, does not have a substructure suitable for product use which is standardized with the legal legislation in terms of food safety and good production practices in production of bee products and where the production stages can be monitored, and the content and the mechanism of action are defined. However, bees and their products, with a very old history, are also mentioned in the holy books. In the "Exit "3: 8 parts of the Bible, while Palestine is

praised, this place is described as the “place for milk and honey”. In the verses 68-69 of “Surah Nahl “, meaning “honeybee” in the Qur’an, with the sayings:” Your Lord reveal to the honeybee: Make homes from mountains, from trees and from pergolas. Then, eat all kinds of fruits, and walk bowing in the paths of your Lord, walk!!” attention is drawn to the importance of honeybees. The lives of these creatures, which are so important for this planet that we are increasingly devastating, have been the subject of many documentaries, and once again it is seen how important they are for a sustainable life and healthy food production. The therapeutic effects of bee products are better understood nowadays when imperialist capital focal points make the planet uninhabitable for these living things. As an alternative to monopoly expensive drugs produced in huge capital pharmaceutical companies, the demand for bee products that societies have been using for thousands of years the apiterapy applications are becoming widespread.

This book shows with the latest scientific evidence how the bee products, particularly propolis, on which the most scientific research has been done, are successfully used in the treatment of some diseases. After the “Regulation on the Traditional and Complementary Medicine Practices” enacted in our country, we think that the interest in the use of bee products in food and health will increase. However, it should be remembered that to process all bee products and especially to be used in the field of health, first we need competent then knowledgeable beekeepers. I hope that this book, written in a simple and understandable style under the content of an EU project related with Apitherapy will be useful to those who love to read and to search.

Prof. Dr. Kemal ÇELİK

Çanakkale, 2024

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Bircan AKPINAR *Turgut KÜÇÜK*
Aydın Beekeeping Association, Aydın-TÜRKİYE

Murat YILMAZ
Alkan ÇAĞLI *Selda MANAV*
Aydin Adnan Menderes University Aydın-TÜRKİYE

Complementary medicine is a method of treatments that are used along with standard medical treatments but are not considered to be as standard treatments. Complementary medicine refers to a group of therapeutic and diagnostic disciplines that exist largely outside the institutions where conventional health care is taught and provided. Types of complementary therapy are aromatherapy, acupuncture, herbal medicine, massage therapy, yoga and apitherapy. In this project, the handbook on Complementary and Supportive Medicine (CSM) consists mainly the apitherapy as the biggest part of the CSM. Honeybees are known for producing and storing honey, or liquefied sugar, as well as building impressively large nests using wax secreted by workers in a particular colony. There are six popular honeybee

products. These include honey, bee pollen, propolis, royal jelly, beeswax and bee venom. The substances that bees secrete, or produce are obtained by collecting, processing, and storing natural substances that man can collect from the hive or directly from the bees (venom). Bee products are natural foods containing substances that are indispensable for life. Basically, all essential amino acids needed by the metabolism, vitamins, minerals, proteins, carbohydrates which are directly assimilated without any processing in the human body, lipids, enzymes, coenzymes, organic acids, etc. are found in bee products.

Bee products have been registered among natural elements used to supplement and improve the food and then to combat and prevent human suffering and pain since the beginning of prehistory. Apitherapy, as a traditional practice dates from immemorial time of human history. Honeybee, venom, bee pollen, raw honey, royal jelly, and propolis are the products generally considered to have medicinal effects. It is important to note that Apitherapy is not only the use of the venom for healing, often called Bee Sting Therapy, but the use of all the hive products, and usually a combination of them. Studies show that bee products may help manage autoimmune diseases, cancer, Alzheimer's, HPV, Lyme Disease, multiple sclerosis, and arthritis bacteria in bee stomachs could even act as alternatives to antibiotics. However, unfortunately severe allergic reactions, and even death, might occur with the use of bee venom or bee related

products. Since apitherapy may involve serious risk of allergic reactions and may even cause death. Apitherapy should only be undertaken after careful thought, and discussion with a qualified apitherapist and your own family doctor.

What is Complementary and Supportive Medicine (CSM)?

CSM is the form of treatments that are used in addition to (complementary) or instead of (Supportive) standard treatments. These practices generally are not considered standard medical approaches. Standard treatments go through a long and careful research process to prove they are safe and effective, but less is known about most types of complementary and alternative medicine. Complementary and alternative medicine may include dietary supplements, megadose vitamins, herbal preparations, special teas, acupuncture, massage therapy, magnet therapy, spiritual healing, and meditation.

Complementary and Supportive medicine (CSM) is a broad domain of healing resources that encompasses all health systems, modalities, and practices and their accompanying theories and beliefs, other than those intrinsic to the politically dominant health system of a particular society or culture in each historical period. CSM includes all such practices and ideas self-defined by their users as preventing or treating illnesses or promoting health and well-being. Complementary medicine refers to a group of therapeutic and diagnostic disciplines that exist largely

outside the institutions where conventional health care is taught and provided. Complementary medicine is an increasing feature of healthcare practice, but considerable confusion remains about what exactly it is and what position the disciplines included under this term should hold in relation to conventional medicine. Types of complementary therapy are:

- Aromatherapy
- Acupuncture
- Apitherapy
- Herbal medicine
- Massage therapy
- Yoga

Complementary medicine is one of the treatment methods that are used instead of standard medical treatments. One example is using a special diet of bee products to treat cancer instead of anticancer drugs that are prescribed by an oncologist. Integrative medicine is a total approach to medical care that combines standard medicine with the CSM practices that have been shown to be safe and effective. They treat the patient's mind, body, and spirit. CSM therapies include a wide variety of botanicals and nutritional products, such as dietary supplements, herbal supplements, and vitamins. Many of these "natural" products are safe because they are present in, or produced by, nature. However, that is not true in all cases. In addition, some may affect how well other medicines work

in your body. For example, the herb St. John's wort, which some people use for depression, may cause certain anticancer drugs not to work as well as they should. All conventional cancer treatments, such as chemotherapy and radiotherapy, must go through rigorous testing by law in order to prove that they work. Most alternative therapies have not been through such testing and there is no scientific evidence that they work. Many health professionals are supportive of people with cancer using complementary therapies. There are some health professionals that have been reluctant for their patients to use them. This is usually because many therapies have not been scientifically tested in the same way as conventional treatments. Research has been carried out to see how well complementary therapies work for people with cancer. And there are some still in progress. But we need more to find out how best to use complementary therapies.

In this project, the handbook on Complementary and Supportive Medicine-CSM will consist mainly the apitherapy as the biggest part of the CSM.

Honeybees And Bee Products

Honey Bees

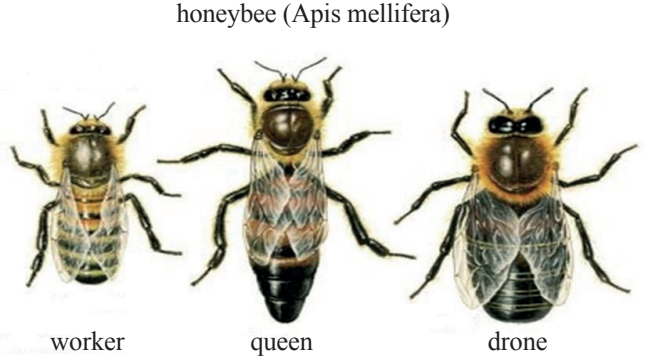

Photo 1. Members of the hive (Encyklopedia Britanicca, Inc., 2006)

The Food and Agriculture Organization of the United Nations (FAO) estimates that of the 100 crop species that provide 90% of food worldwide, 71 are pollinated by bees. Most crops grown in the European Union depend on insect pollination. Beyond the essential value of pollination to maintain biodiversity, the global annual monetary value of pollination has been estimated at hundreds of billions of euros. Aristotle called honey the nectar of the gods. Throughout history, raw honey, bee pollen, propolis, and royal jelly have been valued as both food and medicine. Science now confirms this ancient wisdom. Honeybees represent only a small percent of bee species. They are the only surviving group of bees from the Apini tribe, which is under the Apisgenus. The honeybee is a member of the insect class Insecta. These insects are members

of the subfamily Apinae, which produce, and store liquefied sugar, otherwise known as honey. The bee belongs to the order Hymenoptera, one of the most advanced groups of insects, characterized by social life and organization of individuals in a family. The bee family functions as a "supraorganism" where breathing, nutrition, reproduction, and defense are found both at an individual and a social level. Honeybees are known for producing and storing honey, or liquefied sugar, as well as building impressively large nests using wax secreted by workers in a particular colony. Honeybees live in hives (or colonies). The main property of a bee family is the division of labor among its members. The bees, as a colony, as a family, live together as 30,000 to 50,000 individuals. For a bee colony to function normally and smoothly for a long time, it needs three components: the queen, several drones, and an "army" of worker bees. All of them can ensure the reproduction of bee larvae. Each member of the family has a certain task during a year, which is indispensable for the continuity of the colony. The members of the hive are divided into three types:

The queen is the only female capable of breeding, mating with drones (it typically mates with up to 10 drones) and laying fertilized eggs (which will come out queens or workers) or unfertilized (which will come out drones). It differs slightly from other bees in shape and size. One queen runs the whole hive. Her job is to lay the eggs that will spawn the hive's next generation of bees. The queen also produces chemicals that guide the behavior of the other bees.

Worker bees are, in size, the smallest individuals of the bee family. They are bee females with undeveloped ovaries and incapable of breeding. Worker bees are the most familiar-looking member of the honeybee hive, as they make up about 99% of each colony's population. These are all female, and their roles are to forage for pollen and nectar from flowers, build and protect the hive, clean, and circulate air by beating their wings. Workers are the only bees most people ever see flying around outside the hive. If the queen bee dies, workers will create a new queen by selecting a young larva and feeding it a special food called "royal jelly **"**The drones are the male bees, and their purpose is to mate with the new queen. Several hundred live in each hive during the spring and summer. But when the winter comes, when the hive goes into survival mode, the drones are kicked out! Their bodies are larger than the bodies of workers and the queen. In the bee family, drones have the role of breeding with the queens, to ensure the perpetuation of the species. As a result of biological peculiarities in their possession, bees differ from other creatures cared for and exploited by man because they coexist in families consisting of many individuals, who are well organized, keeping the unity of the bee family.

Photo 2. Queen bees

Photo 3. Drones

Bee Products and Their Uses

The substances that bees secrete or produce, are obtained by collecting, processing, and storing natural substances that man can collect from the hive or directly from the bees (venom). Bee products are natural foods containing substances that are indispensable for life. Basically, in bee products all essential amino acids needed by the metabolism, vitamins, minerals, proteins, and carbohydrates are directly assimilated without any processing in the human body and lipids, enzymes, coenzymes, organic acids, etc are found in bee products. But of all existing foods on earth, honey has the chemical formula closest to that of human blood. Moreover, no other food is more complete, better tolerated and more easily assimilated by the body. Therefore, if a complete classification of biological food would be made, bee products would occupy the first place, not only because of their content (a real fuel for the body), but also because they can be harnessed by the human body without any effort, with resonance at the cellular level, the best regenerative cells. There are six popular honeybee products. These include honey, bee pollen, propolis, royal jelly, beeswax and bee venom.

Honey is made from the nectar collected by bees from many different flowers. The bees store the nectar in their hive, in the concentrated form of honey, mainly for their own food. Honey is a good source of energy, because of its carbohydrate content. It is a good source of vitamins and various minerals. It has a mild antibacterial and antimicrobial activity. Bee pollen is collected by the worker bees from flowers and is used as the protein part of their diet. Bee pollen is an energy stimulating nutritional supplement. Bee pollen contains various vitamins, minerals, fatty acids, and protein. But the quantity of these nutrients is not more than that is seen in many food items. People use bee pollen as a multivitamin, energy booster, and/or to build up their resistance to air-borne (hay fever type) allergens.

Propolis is a sticky resin that seeps from the buds of certain trees and is also called bee glue. Worker bees collect this glue and mix with their salivary secretions to create the glue that is used to coat the inside of the hive. Propolis is a combination of beeswax, honey, and tree resins mixed with bee-produced enzymes, used to protect the beehive from bacteria, fungus, and viruses. Royal jelly is the milky white waxy substance produced by the salivary glands of the worker bees. This is the food for the larval bees in the colony. The queen bees are fed this during the whole of their larval period, but worker bees are fed this for the first three days of their larval period only. This encourages correct development, and the secret of bee queen long life is believed to be related to

consumption of royal jelly. It is also believed to cause increased fertility in the queen bee. Bee wax is secreted by the worker bees from their glands on the underside of her body and used to build the home in which the bees live. This wax is created from the honey that these bees consume. Bee Venom is made by bees. This is the poison that makes bee stings painful. Bee venom is used to make medicine.

What is Apitherapy/ Bee Therapy?

The term Apitherapy comes from the Latin apis, which means "bee." Apitherapy, or bee therapy, is the use of products of the common honeybee for therapeutic purposes. The history of apitherapy extends back to ancient Egypt, Greece, and China. Even Hippocrates, the Greek physician known as the "father of medicine," used bee venom to treat arthritis and other joint problems. Austrian physician Phillip Terc initiated the modern study of bee venom and intentional bee stings when he published his article "Report about a Peculiar Connection Between the Beestings and Rheumatism" in 1888. The late beekeeper Charles Mraz of Middlebury, Vermont, is credited with popularizing bee venom therapy over the past 60 years in the United States. Bee products have been registered since the beginning of prehistory among natural elements used to supplement and improve the food and then to combat and prevent human suffering and pain. Apitherapy, as a traditional practice dates from immemorial time of human history. Today, there are many societies for apitherapy in the world.

There is a commission for apitherapy at the Apimondia, the International Federation of Beekeepers' Associations, which claims to promote scientific, ecological, social, and economic apicultural development in all countries and the cooperation of beekeepers' associations, scientific bodies, and of individuals involved in apiculture worldwide.

According to Dr Stefan Stangaciu, editor in chief of the International Federation of Beekeepers' Association, apitherapy is, 'the art and science of treatment and holistic healing through the honeybee and her products for the benefit of mankind and all the animal kingdom'. The roots of apitherapy can be traced back more than 6000 years to medicine in ancient Egypt. The Greeks and Romans also used bee products for medicinal purposes. This is described by Hippocrates (460–370 BC), Aristotle (384–332 BC) and Galen (130–200 AD), who prescribed the use of honey and bee venom as a cure for baldness. However, whether these practitioners from the ancient world really represent the fathers of apitherapy is questionable. Honeybee venom, bee pollen, raw honey, royal jelly, and propolis are the products generally considered to have medicinal effects. It is important to note that Apitherapy is not only the use of the venom for healing, often called Bee Sting Therapy, but the use of all the hive products, and usually a combination of them. These products are also sometimes mixed with other ingredients, specifically different essential oils, dependent on the condition being treated. These products are said to be effective against a wide range of ailments, from

arthritis and chronic pain to multiple sclerosis and cancer, although few scientific studies have yet proved their benefits. Many different products created by bees can help treat different conditions and offer many different benefits.

They:

- help fights against pathogenic microorganisms.
- improve appetite and the digestive system.
- improve metabolism of human tissues.
- reduce fat accumulation.
- regulate bowel function in constipation and pollen has a radioprotective and anti-tumor effect.

Studies show that bee products may help manage autoimmune diseases, cancer, Alzheimer's, HPV, Lyme Disease, multiple sclerosis (MS), and arthritis bacteria in bee stomachs could even act as alternatives to antibiotics. Primary care physicians are generally concerned about complementary/supportive treatment. But the World Health Organization (WHO) recommends honey with its antimicrobial characteristics as an appropriate method for cough and cold. Honey as a remedy is for example popular for upper respiratory tract infections in Germany, Norway, Spain, Venezuela, and the Middle East. And it is used traditionally as a remedy for a long time in India, Nigeria, and Ghana.

Epidemiological studies and animal experiments report on the utility of pollen in prostate hyperplasia and allergic disease, bee venom for mildering pain in rheumatic

disease and in controlling multiple sclerosis attacks, propolis in cardiovascular disease and royal jelly in providing the flowability of red blood cells. There is a major difference between apitherapy and the use of bee products in defined medical situations. Apitherapists believe that bee products can be used to cure most diseases. However, the use of bee products in conventional medicine is limited to certain indications where they have shown effects which are equal to or better than those of standard treatments - for example, in treating wounds and burns and as an interesting approach in arthritis. In the health field, a properly dosed combination of honey, pollen, and royal jelly, has a very important role. This combination is used for mother and child care, adult health, recovering, vitamin deficiency, various diseases of the digestive tract and liver, in respiratory disorders, neurosis, asthenia, and senility. Recommendations towards using apitherapy should be made only because of precise medical diagnosis, laboratory tests, radiological and other investigations required. However, of all bee products, bee venom has the best and oldest therapeutic use, with the purpose of treating rheumatic and joint pain, chronic inflammatory diseases (tendinitis, bursitis) and Multiple Sclerosis. In the early 1950s, studies carried out worldwide have led to a better understanding of the qualities traditionally attributed to honey and propolis. In addition, they found benefits, until then unknown, of pollen and royal jelly. Cosmetics, based on bee products, positively influence physiological functions of skin cells, regenerate, protect the skin from free radicals and harmful influences of the

environment, adjust metabolism, stimulate collagen production, delay degenerative changes, and increase defense power. They also provide substances for skin reconstruction and they visibly improve the structure, elasticity, color, skin suppleness and smoothness. Cosmetics based on bee products are ideal for prevention of skin aging phenomena. Honey, beeswax, and propolis are used as healing and pampering agents in a few body care products including soap, lip balm, cremes, salves and lotions.

Why Do We Need Apitherapy?

Bees contribute to peoples' livelihoods in almost every country on earth. Honey and the other products obtained from bees have long been known by every society. In many parts of the world, significant volumes of honey are today still obtained by plundering wild colonies of bees, while elsewhere beekeeping is practiced by highly skilled people. Beekeeping is an ancient tradition, and honeybees have been kept in Europe for several millennia. Bees contribute to human wealth and wellbeing directly through the production of honey and other food and feed supplies such as: pollen, wax for food processing, propolis in food technology, and royal jelly as a dietary supplement and ingredient in food.

The main reasons for Apitherapy are:

- Preventing diseases when they are consumed on a regular basis; as opposed to chemical treatments, bee products don't have side effects when properly used.

- They are extremely rich in nutrients and active compounds which can protect the human body against various diseases.
- The bee products have an extraordinary richness in nutrients and "soft" active compounds which can protect our health efficiently against over 500 diseases.
- The beekeepers have the second highest longevity among all professions; they are usually strong and generous with their friends all their life; why?

Apitherapy uses beehive products (honey, pollen, wax, propolis, royal jelly, etc.) in treating a wide range of conditions, which cover the entire body since the dawn of history. The benefits of treatments based on materials collected and processed by bees passed from empirical to scientific medicine process which confirmed the undeniable value of bee products in healing various diseases.

Apitherapy Applications

Ease arthritis pain- Bee venom therapy (BVT) has been used since ancient Greece to help relieve pain from rheumatoid arthritis. This is due to its anti-inflammatory and pain-relieving effects. Research has found that BVT can lead to a decrease in swelling, pain, and stiffness in people with rheumatoid arthritis. One study even found that it can reduce the need for traditional medications to be used, and that

it simultaneously reduced the risk of relapse.

Heal wounds- Honey has long been used topically to treat wounds including both open cuts and burns thanks to its antibacterial, anti-inflammatory, and pain-relieving properties. Today's research backs this up. A 2008 review found that medical dressings containing honey were effective at helping heal wounds while lowering the risk of infection.

Helps with allergies. Local wildflower honey, as it turns out, can help treat allergies in several ways. Honey can soothe a sore throat caused by allergies and act as a natural cough suppressant. Local wildflower honey may also protect people from allergies. This is because local wildflower honey can also contain trace amounts of flower pollen, a known allergen. Consuming local honey could slowly introduce this allergen to the body, potentially building up immunity to it.

Treat immune and neurologic conditions. BVT can be used as a complementary treatment for diseases tied to both the immune system and the neurologic system, including: Parkinson's disease, multiple sclerosis, Alzheimer's disease, lupus.While bee venom shouldn't be the first or only method of treatment for these conditions, research found evidence that bee venom was able to boost the immune system and reduce some symptoms of these conditions in the body- partially thanks to bee venom's anti-inflammatory effects.It's important to note that this research also indicates that bee venom can be a double-edged sword. Bee venom can cause side effects in many people. Regulate thyroid function- BVT was

found to help regulate thyroid function in women who have hyperthyroidism. However, research into BVT as a thyroid treatment is currently very small, and more studies are needed.

Reduce gingivitis and plaque. Propolis can have several health benefits. It can reduce gingivitis and plaque when it's added to a mouth rinse. Research into propolis-containing mouthwashes found that it may be able to naturally protect against oral diseases. Propolis may even help heal and prevent canker sores as well.

Serve as a multivitamin. Both royal jelly and propolis contain many vitamins and nutrients. They can be taken as multivitamins to improve overall health, including hair appearance. Propolis is available as an oral supplement and an extract. Royal jelly can be found in soft gel and capsule form.

Medicinal Properties of Bee Products

Apitherapy is the use of products from the bee to heal a variety of medical conditions and promote health. The products that are usually used include bee venom, bee pollen, raw honey, royal jelly, and propolis.

Bee Venom is most popular for the treatment of multiple sclerosis and many forms of arthritis. Bee venom is administered to patients either through direct bee sting or by injections. Various studies have shown that bee venom contains various substances including, adolapin and melittin. These compounds are very potent chemicals with antiinflammatory activity exceeding those of steroids. Melting also stimulates

the production of cortisol in the body, which is a natural steroid compound possessing anti-inflammatory properties. Because of its anti-inflammatory property, bee venom is used in various conditions that has inherent inflammatory processes, like tendonitis, bursitis, and arthritis, including rheumatoid arthritis and osteoarthritis. The only condition that has actual scientific data supporting the use of Apitherapy for treatment is post-herpetic neuralgia. There are some reports that suggest that it may be useful in the treatment of infectious, auto-immune, cardiovascular, pulmonary, gastrointestinal, neuropathic pain and other chronic pain conditions. The venoms' benefits over a longer period. It is inert, i.e. it does not human beings as well as to animals are very long. Most interact with the human digestive system at all and pass the reports of cures of individual cases, though through the body unaltered. However, substances several unrelated patients have experienced dissolved or encapsulated in waxes are slowly released to improve or cure of similar ailments.

Some apitherapy practitioners use **bee pollen** to treat seasonal allergy, because ingestion of small amounts of pollen may desensitize the patient. There are various claims about the benefits of bee pollen. Many of these claims including its potential to improve performance of athletes, and anti-aging activities are not yet supported by scientific evidence.

Honey is a good source of energy, because of its carbohydrate content. It is a good source of vitamins and various minerals. It has a mild antibacterial and antimicrobial activity.

Honey is shown to soothe sore throat. Apitherapist uses raw honey which has not been filtered, heated, or processed in any form. Raw honey is shown to be better than processed honey in some studies. Raw honey is used in apitherapy to suppress bacterial and microbial infections, especially those associated with skin wounds.

Royal jelly has been used in a variety of medical conditions, including fatigue, infertility, lack of appetite and asthma. There are many clinical reports of the benefits of royal jelly in a variety of other medical conditions, but these claims are not largely supported by clinical studies. Animal and human studies have shown that royal jelly is capable of lowering cholesterol levels. Royal jelly is often used in women's cosmetics including wrinkle creams. There is no scientific evidence to support the claim that royal jelly retards the aging process in human beings.

Propolis has natural anti-bacterial, anti-viral, anti-fungal, antioxidant, and anti-inflammatory properties. People use propolis as a remedy for colds & influenza, and to boost the immune system. The antibacterial and antifungal properties of propolis make it an ideal topical ingredient for treating various skin conditions. Propolis also serves as a source of flavonoids, which is a potent antioxidant. Antioxidants are shown to heal the injury to damaged cells.

Bee wax is used in face and hand creams, ointments, lipsticks and lip salves, coating tablets and capsules in the pharmaceutical industry.

Precautions for Bee Products in Apitherapy

Since apitherapy may involve serious risk of allergic reactions and may even cause death. Apitherapy should only be undertaken after careful thought, and discussion with a qualified apitherapist and your own family doctor. The therapy should be carefully monitored for any adverse events. Do not attempt to collect bees and start stinging as this may prove catastrophic. Severe allergic reactions and even death may occur with the use of bee venom or bee related products. Anyone attempting this therapy is doing the therapy at his or her own risk. Therapy with other type of bee products usually does not require the supervision of a trained apitherapist. Bee pollen and royal jelly are available over the counter in many forms including capsules, powders, cream, and lotions for internal and external uses. Raw honey and propolis are available in many health-food stores. Any of the honeybee precuts may cause allergic reaction, hence care must be taken when initiating treatment with these group of compounds.

The only contraindications of apitherapy are age (<1 years), and the presence of bee and bee product allergy.

A guideline developed by Dr Stangaciu is summarized in below:

- Before starting apitherapy, one must ‘clean’ the body with different ‘detoxifying’ methods: special diets, fasting, colon cleansing if necessary.

- The fresh, 'organic' bee products have usually better effects than the 'industrial' processed ones; over-heat, excessive filtration and refining are detrimental.
- Select attentively the bee products according to their origin, composition, and pharmacological properties.
- The quality and methods of storage are most important for good efficiency.
- Apply with flexibility the producer's (manufacturer's) recommendations.
- Always test for allergies before you start the treatment.
- Gradually increase the doses of bee products.
- Use several 'vehicles' to better reach the affected area: liquids (tea, water, juices); creams/ointments; inhalations; suppositories, injections etc.
- Several methods of administration are better than only one.
- The dose of each bee product must be established with accuracy according to the age, weight, general/local condition of each patient, time of application etc.
- 'Simillia simillibum curantur': small doses can be used to treat bee product allergies (as in pollen, bee venom and honey allergies).
- The time of treatments should be in harmony with different (bio) rhythms; these rhythms vary with the patient, the disease, the season, the hour of the day etc.
- Apitherapy is not a 'panacea' and should be applied in harmony with other natural healing methods like Phytotherapy, Aromatherapy, Acupuncture, Organic diet, Ayurveda, etc.

- ‘Primum non nocere’! Do not experiment on your patient! Use only safe methods and high-quality products!
- It is very important to improve the blood flow through other methods like Massage, Acupressure, Gymnastics, Taiji Quan, Qigong, Hatha Yoga etc.
- Good sleep and relaxation enhance the effect of bee products.
- Good environment (clean, ordered, and non-polluted) and a ‘positive thinking’ family/friends’ group are also beneficial.
- Individualize your treatment! Each patient is Unique and must receive a unique treatment!
- Because of their composition, all bee products have beneficial effects on all patients.
- Apitherapy is not a ‘blitz’ method! Perseverance and patience is necessary, especially in chronic diseases.
- Educate your patients before, during and after treatments; make them true bee lovers and protectors! Each patient must become, in time, his own apitherapist.
- A good apitherapist must know the bee colony’s life in detail; he must be also at least a good ‘amateur’ beekeeper.
- Continuous study, good exchange of information with other specialists from several ‘Apitherapy related countries’, regular use of Internet can help in finding the best medical strategy for each person.

Be careful with bee venom therapy (BVT)

BVT can be dangerous. Bee venom can induce a histamine response. This can cause anything from irritation like swollen, reddened skin to severe allergic reactions that can be life-threatening. BVT can be painful. Even if you aren't severely allergic to bees, it could still lead you to experience negative side effects. These include headache, cough, uterine contractions, discoloration of the sclera, or white of the eye, jaundice, or yellowing of the skin, severe pain in the body and muscular weakness.

- It is the most dangerous of all bee products.
- This treatment should be undertaken only under the care of a trained apitherapy practitioner.
- It is important to test the patients for allergies before applying any medicinal bee venom treatment.
- Once the safety of bee venom is established for an individual, the treatment may be done at home. The venom is administered through injection or through bee sting.
- If the bee sting is used the apitherapy practitioner will place bees on the skin, typically close to the joints, muscle or other body parts that are having problems.
- Apitherapy using bee sting may be painful, but these are not very painful as wasp or hornet stings.
- The treatment may be followed by local discomfort, inflammation, stiffness, and soreness or itching.
- Usually, an ice pack treatment is given after the bee stings to reduce these side effects.

Check Yourself

1. **What CAM doesn't include?**
   a) bee products
   b) acupuncture
   c) herbal supplements
   d) drugs

2. **Which one isn't a member of honey bees family?**
   a) queen
   b) drone
   c) butterfly
   d) worker bees

3. **What CAM doesn't include?**
   a) bee products
   b) acupuncture
   c) herbal supplements
   d) drugs

4. **Which product can't be used for apitherapy?**
   a) sugar
   b) honey
   c) royal jelly
   d) bee venom

5. How can bee products help treat different conditions?

a) helps fight against pathogenic microorganisms.
b) improve appetite and the digestive system
c) improve metabolism of human tissues
d) all

6. Which isn't a way of use for bee wax?

a) face and hand creams,
b) ointments,
c) antibiotic
d) lipsticks,

7. What is the most dangerous bee product for apitherapy?

a) bee venom
b) royal jelly
c) honey
d) propolis

8. The only contraindication of apitherapy are age:

a) 5 years
b) <1 years
c) 4 years
d) 2 years

9. What does bee venom induce?

a) antibiotic response
b) antifungal response
c) antibacterial response
d) histamine response

10. Which one is the treatment after the bee stings?

a) icepack treatment
b) antibiotic treatment
c) hot water treatment
d) antifungal treatment

Answers: 1d, 2c, 3d, 4a, 5d, 6c, 7a, 8b, 9d, 10e

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HONEY

Prof. Dr. Kemal ÇELİK Prof. Dr. Harun BAYTEKIN
Çanakkale Onsekiz Mart University, Çanakkale, TÜRKİYE

Honey - What's it and How Is It Made

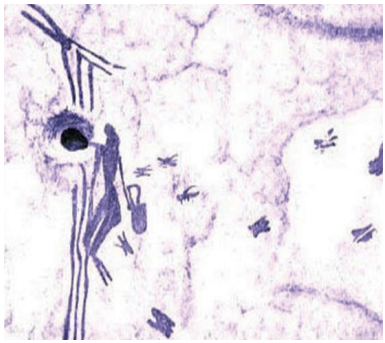


Photo 4. Prehistoric man gathering honey -rock painting (6000 BC)

Honey has been an important food for men since the very beginning of mankind. As far as back 7000 BC people gathered and consumed honey that was the only available sweet food product. The oldest rock painting dated in 6000 BC founded in Cueva de la Arana, near Valencia in Spain shows a prehistoric man gathering honey. Wound healing was probably the first use of honey for human health. Sumerian clay tablet writing, dating from 2100-2000 BC are the first written reference to honey that describe it as a drug and ointment. Some prescriptions for healing wounds with honey are also mentioned there. According to

the Smith papyrus (1700 BC) it was used in wound healing while the Ebers papyrus (1550 BC) indicates honey as the remedy for spotted baldness or anti-inflammatory agent. Honey was also mentioned numerous times in prescriptions and medical indications (mostly as cure for wounds healing, against different internal and external infections), in ancient Chinese, India (ayurvedic medicine), Egypt, and ancient Greek medicine. An Ancient Chinese prescription book found on a silk scroll in 3rd century BC near Changsha in Hunan province consists of fifty-two prescriptions, including one prescription with honey used to treat diseases. Honey is also reported in some authoritative religions as a health-promoting food. In the Bible, the wise king Salomon says:" Eat honey my son, because it is good" (Old Testament, proverb 24:13). The Koran says "thy Lord taught the bee to build its cells in hills, on trees and in (men's) habitations their issues from within their bodies a drink of varying colors, wherein is healing for mankind (Quran 16:68-69). Muhammad al-Bukhari (810–870) - Sunni Islamic scholar

Photo 5. Preparation of honey medicine from De Materia Medica, Dioscorides (in Arabic), 1224

from Bukhara who wrote Sahih al-Bukhari - hadith collection that is the second important religious book after the Koran, quoting the Mohammed the Prophet (571-632 AD), wrote: ***"Honey is a remedy for every illness.***

Honey is a sweet product made by honey bee (*Apis mellifera*) and subspecies such as *A. mellifera caucasica, A. m. carnica, A. m. anatolica or other species such as A. andreniformis, A. caucasica, A. cerana, A. dorsata, A. florea, A. indica, and A. ligustica*; *Plebeia wittmanni, Tetragonisca angustula fiebrigi*, and *Trigona carbonaria* from mono-multiflower nectar, combined with an bee's enzyme, then evaporated to reduce water content in the honeycomb cells. Honey is a sweet and flavourful natural product, rich in sugars, and with high nourishing value. There are also some minor components present in honey, such as polyphenols, vitamins, minerals, enzymes (glucose oxidase and catalase), carotenoids, amino acids, proteins, organic acids, and volatile compounds. Due to the oligosaccharide's presence honey demonstrates prebiotic effects. Honey's composition clearly depends on the floral source, geographical region, and season, as well as the processing conducted after the harvest. Today, approximately 300 types of honey have been recognized (Bogdanov 2011, Viuda-Martos *et al.* 2008). Total production of honey was almost 1.9 million tonnes in 2017. Around one/third of global honey production took place in China other significant producers was Türkiye, Iran, United States, and Ukraine,

An average bee colony produces 27 - 45 kg of honey per year. There are three types of bees in the colony: 50000-70000 workers, one queen bee, and 2000 drones. Work-ers live for three to six weeks, collecting during their life of about one teaspoon of nectar. Production of one kilogram of honey requires collection of 3,5 kg of nectar. To collect such an amount of nectar four million flowers are needed. Worker bees leave the hive to collect nectar when they are about 20-day old. Workers collect nectar by sucking it through its proboscis and placing it in its proventriculus (honey stomach or crop), which lies just dorsal to its food stomach. The honey stomach can hold about 50% of the bee's weight (40 mg of nectar). Salivary enzymes and proteins from the bee's hypopharyngeal gland are added to the nectar to begin breaking down the sugars. Bee digestive enzymes hydrolyze sucrose to a mixture of glucose and fructose, and break down other starches and proteins, increasing the acidity. During foraging pollen attaches to bees' legs and mixes with the nectar. When workers' sacs are full, bees go back to the hive. Nectar is transported to the indoor bees and passed mouth-to-mouth from bee to bee to reduce moisture content and storage in honeycomb's cells. Sometimes the nectar is stored at once in cells of the honeycomb because some evaporation occurs due to 32.5°C temperature inside the hive. The nectar becomes honey when moisture content is reduced from about 70% to around 17%. The honey is placed in honeycomb's cells and sealed with beeswax. Stored honey is used by adult

and larval bees as food when other food sources are scarce or during the cold weather.

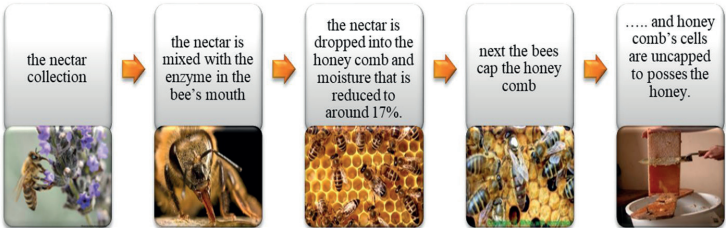


Fig. 1. Formation of honey

HONEY CHEMICAL AND PHYSICAL CHARACTERISTICS

Physical Characteristics of Honey

Honey rheology – viscosity, density, hygroscopicity, and surface tension

Water content (%)	Specific gravity at 20°C	Water content (%)	Specific gravity at 20°C	Water content (%)	Specific gravity at 20°C
13.0	1.4457	16.0	1.4295	19.0	1.4101
14.0	1.4404	17.0	1.4237	20.0	1.4027
15.0	1.4350	18.0	1.4171	21.0	1.3950

Table 1. True specific gravity of honeys with different water content (White, 1975)

Raw, fresh honey is a thick, viscous liquid. Honey **viscosity** mainly depends on water and carbohydrate contents. The viscosity of honey is 10,000 cP at a room temper-ature of 21.1°C. Honeys vary with viscosity manuka and heather honeys

are extremely vicious and there are defined as thixotrophic and their viscosity decreases with agitation. Conversely, viscosity of eucalyptus honey increases when stirred or agitated. The viscosity of honeys decreases together with increasing temperature.

Another physical characteristic of practical importance is density. Honey **density**, expressed as specific gravity is greater than water density, but it also depends on the water content of the honey. Because of the variation in density, it is sometimes possible to observe distinct stratification of honey in large storage tanks. The high-water content (less dense) honey settles above the denser, drier honey. Such inconvenient separation can be avoided by more thorough mixing. Honey density (expressed as specific gravity) is higher than density of water is strictly correlated with water content in honey.

Sample no.	Density at20°C	Density at 26,5°C	Density at 35,9°C
1	1.472	1.464	1.448
2	1.490	1.479	1.461
3	1.469	1.461	1.441
4	1.487	1.472	1.451
5	1.499	1.486	1.469
6	1.462	1.457	1.444

Table 2. Honey density at three different temperatures (Mehryar at al. 2013)

This physiological parameter has the great practical significance. Due to discrepancy in various honeys density,

during the honey storage in large containers, honey stratifies – the honey with lower moisture content settles under the less dense honey (with higher moisture). To avoid undesirable stratification honeys with various water content should be thoroughly mix. Besides moisture content, the temperature affects honey density. The higher the temperature, the lower honey density.

Air (%RH)	Honey (% moisture content)
50	15,9
55	16,8
60	18,3
65	20,9
70	24,2
75	28,3
80	33,1

Table 3. Approximate equilibrium between relative humidity (RH) of ambient air and water content of a clover honey (White, 1975)

Honey has hygroscopic properties, meaning that it absorbs water from things, even from the air. Due to this feature, it provides almost no water for growth of mi-crobes. It makes it a good material for open wound healing. It keeps the wound moist, promotes new tissue formation, and prevents dressing from sticking to the skin. Honey is also a good constituent in numerous cosmetics products that protects skin from drying and moisturizes it. Standard honey with a moisture content of <18.3 % ab-sorbs moistness from the air at a relative humidity

of above 60%. Honey has rather low **surface tension** of 50–60 MJ/m2. Depending on origin, honeys vary with surface tension. The high viscosity and wettability of honey results in stickiness. Thanks to low surface tension, honey is very good humectant in cosmetics.

Phase transitions

The melting point of crystallized honey varies from 40 and 50 °C, depending on its chemical composition. In lower temperature, honey's physical form is usually labile and crystallizes spontaneously through saturation by sugars. It could also be in a stable state and crystalize after adding the seed crystal. There are many factors affecting honey crys-tallization rate and the most important is sugars content, especially fructose to glucose ratio. Honey with high content of glucose such as canola or dandelion honey crystallized in a very short time after harvesting. On the other hand, chestnut or tupelo honeys that are characterized with low percentage of glucose do not crystallize. The process of honey crystallization is also influenced by moisture content - the higher the water content is, the lower rate of honey crystallization. Temperature has been proved to have an effect on honey crystallization too. The fastest growth of the rate of crystallization has been found when temperature is from 13 and 17 °C. The size of crystals forming in the crystallization process also depends on temperature. Bigger but less numerous tend to form in higher temperature whereas smaller and numerous ones generally

form in lower temperature. Honey does not crystallize below 5 °C. Honey does not freeze solid, even in very low temperatures but its viscosity increases, and honey becomes thicker. A glass transition point for honey when it becomes solid and ranges from −42 to −51 °C.

Chemical Characteristics of Honey

Honey consists of 16 - 18% of moisture. Honey is protected from microbial con-tamination and can be deposited for a long period at room temperature without any preservative application due to low moisture content and high osmotic. However, due the presence of osmophilic yeast, fermentation may occur (Bhandari *et al.* 1999). On a dry matter basis the main constituents are sugars and in lower quantities proteins, amino acids, organic acids, enzymes, minerals, vitamins, polyphenols, and volatile substances. Fructose and glucose contribute up to 75% of total carbohydrates included in honey. The average fructose content is 39% while glucose – 31%. Generally, the most abundant honey's sugar is fructose, especially in acacia honey (*Robinia pseudoacacia*) that contains the highest quantities of this monosaccharide. However, there are some types of honey such as rape honey (*Brassica napus*) and dandelion honey (*Taraxacum officinale*) that contain more glucose than fructose (Persano Oddo, 2004). Besides the main monosaccharides in honey, some oligosaccharides including sucrose, isomaltose, maltose, maltulose, turnose, trehalose, panose, palatinose, 6-kestose,

1-kestose, malto-triose, melezitose and others are contained in honey (Bogdanov *et al.* 2008).

Minerals [in mg/100 of honey]	
potassium (K)	**40 - 3500**
calcium (Ca)	3 - 31
phosphorus (P)	**2 - 15**
sodium (Na)	1,6 - 17
magnesium (Mg)	**0,7 - 13**
iron (Fe)	0,03 - 4
zinc (Zn)	**0,05 - 2**
copper (Cu)	0,02 – 0,6
manganese (Mn)	**0,02 - 2**
chromium (Cr)	0,01 – 0,3
selenium (Se)	**0,002 – 0,01**

Table 4. After Bogdanov et al. 2008

Proteins (0, 25– 0,5%) are present in honey mainly as enzymes. The major enzymes of honey are diastase (or amylase), invertase (or sucrase, or α-glucosidase) CAT, and glu-cose oxidase as well more than 20 amino acids, among which proline is the most abun-dant. All of nine essential amino acids occur in honey and all nonessential amino acids except for asparagine and glutamine.

The **mineral** content in honey varies from 0, 04% in light honeys, to 0.2%,

In dark honeys. Among all minerals, the most abundant element is potassium. There are other macro and microelements present in honey such as magnesium, calcium, iron, phosphorus, sodium, manganese, iodine, zinc, lithium, cobalt, nickel, cadmium, copper, chromium, selenium, arsenic, and silver.

Other minerals [in mg/100 of honey]			
aluminium (Al)	**40 - 3500**	**lead (Pb)**	**0,001 – 0,03**
arsen (As)	3 - 31	lithium (Li)	0,225 – 1,56
barium (B)	**2 - 15**	**molybdenum (Mo)**	**0 – 0,004**
bromine (Br)	1,6 - 17	nickel (Ni)	0 -0,051
cadmium (Cd)	**0,7 - 13**	**rubidium (Rb)**	**0,04 – 3,5**
chlorine (Cl)	0,03 - 4	strontium (Sr)	0,04 – 0,35
cobalt (Co)	**0,05 - 2**	**silicium (Si)**	**0,05 - 24**
floride (F)	0,02 – 0,6	suflur (S)	0,7 -26
iodine (I)	**0,02 - 2**	**vanadium (V)**	**0 – 0,013**

Table 5. After Bogdanov et al. 2008

Honey also contains small quantities of **vitamins** from B group such as: thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (H), fo-lic acid (B9), and vitamin C. Honey contains 0,3-25 mg/kg choline that is crucial for brain and cardiovascular functions as well 0.06 to 5 mg/kg acetylcholine that play a role of neurotransmitter. Average **organic acid** content in honey is around 0.57%. The most abundant in honey is gluconic acid. There are also other

organic acids occurring in honey in smaller quantities such as aspartic, butyric, citric, acetic, formic, fumaric, galacturonic, gluconic, malonic, propionic, pyruvic, succinic, formic acid and others. These organic acids are responsible for the honey acidity (pH between 3, 2 and 4,5).

The chemical diversity of **phenols** in honey is highly dependent on the floral and geo-graphical origins of honey. However, certification of honey floral origins based exclusively on phenols is not sufficient. Honey polyphenols can be classifying in two groups: phenolic acids (e.g. caffeic, ferulic, gallic, syringic, ellagic, hydroxybenzoic and chlorogenic) and flavonoids (e.g. quercetin, kaempferol, myricetin, pinocembrin, galangin, hesperetin). Recent evidence has shown the occurrence of approximately thirty various polyphenols in honey. The presence and levels of polyphenols in honey may vary depending on the floral source, the climatic and geographical circumstances. Kaempferol, luteolin, quercetin, and galangin are found in all types of honey while hesperetin and naringenin are present only in specific varieties. There are some reports giving evidence for using the phenols as biomarkers for various honey - quercetin and kaempferol for sunflower and rosemary honeys (Tomás-Barberán *et al.* 2001) or methyl syringate and

lumichrome for manuka honey (Oelschlaegel *et al.* 2012). However, Petrus *et al.* (2011) considered quercetin and kaempferol not to be acceptable biomarkers due to their high levels in rape, pumpkin, melon, and cherry blossom

honeys. Also, for evidence of origin of manuka honey, it was reported that these biomarkers are also present in sage, thistle and cornflower honey (Tuberoso *et al.* 2012, Kús *et al.* 2014). Usually, the volatile compounds in honey are low.

They include alcohols, aldehydes, ketones, hydrocarbons, py-ran, terpene and its derivatives, acid esters, benzene, and its derivatives, norisoprenoids, as well as furan, and cyclic compounds (Bogdanov, 2008).

Honey Nutritional and Therapeutic Properties

Nutritional value of honey

According to human standard nutrition, honey should not be considered as a complete food but as a food supplement. The fructose and glucose of honey can be quickly utilized as an instant energy source soon after digestion by the human body. Honey provides 64 calories energy per tablespoon, as fuel for working muscles. It is a source of simple carbohydrates.

The average composition is 17.1% water, 82.4% total carbohydrate and 0.5% proteins, amino acids, vitamins, and minerals. Among carbohy-drate the most abundant sugars are mainly fructose (38.5%) and glucose (31%). The remaining 12.9% of carbohydrates is made up of maltose, sucrose, and other sugars. Honey is fat-free, cholesterol-free, sodium and gluten-free food.

Nutrition Facts		
serving size, 100 g		
nutrients		
amoaunt per serving		**% daily value**
calories	305	15 %
total fat,	0 g	0 %
saturated fat,	0 g	0 %
cholesterol,	0 mg	0 %
sodium, 4 mg		0 %
potassium,	52 mg	1,5 %
total carbohydrates,	82,4 g	27 %
dietary fibre,	0,2 g	-
sugars,	82 g	-
protein,	0 g	0%
vitamins		
riboflavin (B2),	0,038 mg	3 %
niacin (B2),	0,121 mg	1 %
pantothetnic acid (B5),	0,068 mg	1 %
vitamin B6,	0,024 mg	2 %
folate (B9),	2 ug	1 %
vitamin C,	0,5 mg	1 %
minerals		
calcium,	6 mg	1 %
iron,	0,42 mg	3 %
magnesium,	2 mg	1 %
phosphorus,	4 mg	1 %
potassium,	52 mg	1 %
sodium,	4 mg	0%
zinc,	0,22 mg	2 %

Table 6. Nutritional properties of honey

One of the most popular products made of honey is mead – "honey wine" probable is the oldest fermented drink dating to 9,000 years ago. Mead is an alcoholic beverage make as the result of the most used Saccharomyces cerevisiae yeast addition to aqueous honey solutions and fermented for weeks or even months. The alcohol content ranges from about 3.5% to more than 20%. It may be still, carbonated, or naturally sparkling; dry, semi-sweet, or sweet. The fermentation can be divided into primary and secondary. The first one usually lasts 1-2 months and is followed by compulsory secondary fermentation that is longer and takes from 6 to 9 months of aging. The duration time of secondary fermentation depends on many issues such as floral origin, sugars and microorganisms' content, water percentage in must, used additive(s), strain of yeast and others. There are numerous varieties of mead. Metheglin is made with herbs e.g. chamomile, lavender, meadowsweet, or spices such as cinnamon, cloves, nutmeg). Melomel is honey wine with fruits. The specific melomel variety is payment made of honey and grape juice. Hippocras is the variety fermented with cinnamon, and sack mead with high concentration of honey. There are also some seasonal varieties such as mulled mead popular at Christmas time, and flavored with spices and various fruits and traditionally warmed. Honey is also used to make mead beer - "braggot".

Therapeutic properties of honey

Research evidence indicates that honey can exert several health-promoting effects such as antibacterial, anti-fungal,

antiviral, anti-inflammatory, antioxidant, antihypertensive, anticancer, immunomodulatory, and hypoglycemic activity and affect the homeostasis of organism positively.

Antimicrobial Activity

There is strong scientific evidence for honey antimicrobial properties. Numerous clinical studies proved that honey application to infected, cutaneous injuries, enhances processes of wound cleansing and healing. Also, the broad-spectrum antimicrobial activity, including antibacterial, antiviral, antifungal, and antimycobacterial properties of honey has been demonstrated in various in vitro studies. It is assumed that antimicrobial activity of honey is attributed to low moisture content, the honey acidity (low pH) due to presence of organic acids such as gluconic acid, osmotic effect due to high sugar concentration.

Fig. 2. Honey, its properties and beneficial impact on organism.

Hydrogen peroxide is one of the most important bactericidal and bacteriostatic compounds occurring in honey. It is generated in honey due to the glucose oxidase enzyme (GOx) - an oxidoreductase that catalyzes the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone. GOx is synthesized by some insects including honeybee and shows antibacterial properties in the presence of oxygen and glucose. The optimum antibacterial activity is demonstrated in fresh, raw, storage in dark, and unheated honey. The activity of hydrogen peroxide is reduced in honey subjected to thermal processing or storage in the light. The activity of non-peroxidic substances e.g. phenolic acids, flavonoids are less susceptible for destroying under the heat and light exposition. Besides, hydrogen peroxidase the most significant antimicrobial agents included in honey are lysozyme, catalase, antioxidants, polyphenols, flavonoids, phenolic acids,

methylglyoxal (generated conversion of dihydroxyacetone during honey maturation), and bee peptides (bee defensin-1, apidaecin, abaecin, and hymenoptaecin). The average honey pH varies from 3,2 to 4,5. The pH of honey is low due to the acid's presence. The main acid is gluconolactone (gluconic acid) - a product of glucose oxidation by the GOx. Its content in honey is around 1%. Other acids such as formic, acetic, citric, lactic, maleic, malic, oxalic, pyroglutamic and succinic have been found in minor quantities. Honeys also have high buffer capacity due to the presence of phosphates, carbonates, and other mineral salts. Honey derived from the floral source of Leptospermum spp. (manuka) has been claimed to have therapeutic advantages over other honeys due to reactive methylglyoxal (MG). MG has been found in a range of foods and beverages, including wine, beer, bread, and honey. The concentration of MG in manuka honeys -139–491 mg x kg−1 is up to 100-fold higher than in conventional honeys - 0.4 to 5.4 mg x kg1. It's demonstrated that manuka honey is effective against a broad range of microorganisms including multi-resistant strains. The antimicrobial factors of honey depending on origin may be classified in three groups: of bee origin organic acids, carbohydrates, peroxidase forming enzymes, bee peptides; of plant origin polyphenols, flavonoids, phenolic acids, methylglyoxal, and of honey storage origin Maillard products (Bogdanov, 2008). In *in vitro* and *in vivo* studies, various types of honeys have been demonstrated to have antimicrobial properties, against numerous bacteria species,

among which the most importance are: *Campylobacter spp, Escherichia coli, E. coli, O157:H7; Haemophilus influenzae, Helicobacter pylori, Klebsiella pneumoniae, Listeria monocytogenes, Proteus sp, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhimurium, Shigella dysenteriae, Staphylococcus aureus, methicillin-resistant S. aureus (MRSA), Streptococcus hemolyticus group B, Streptococcus mutans, Streptococcus pyogenes, Yersinia enterocolitica*, and others.

Antioxidant Activity

Chronic diseases such as hypertension, cancer, cardiovascular system diseases, atherosclerosis, and Alzheimer's disease are the major causes of death in the world. These diseases are strictly associated with oxidative stress of organisms and their prevalence recently have been in the interest of scientists and the whole society. The detrimental imbalance between oxidants and antioxidants in favor of the oxidants is referred to as oxidative stress. The chronic diseases are susceptible to oxidative stress due to raised oxidant levels and/or not sufficient supplies of antioxidants, usually being the result of imbalanced diets. To prevent, remove or delay oxidative stress there is a need for diet supplementation with food materials rich in antioxidants (Albright, 2008).

The result of oxidative stress is oxidative damage caused by reactive species upon the organisms and in consequence impairment of cellular physiological functions. Reactive

species can be reactive oxygen species (ROS) or reactive nitrogen species (RNS) are formed by aerobic organisms as by-products of metabolism such as for instance mitochondrial electron transport chain or as the consequence of chemistry accidents e.g. autoxidation of unstable biomolecules. Reactive species can also be synthesized by phagocytes as the response to inflammation process (Halliwell and Gutteridge, 2007).

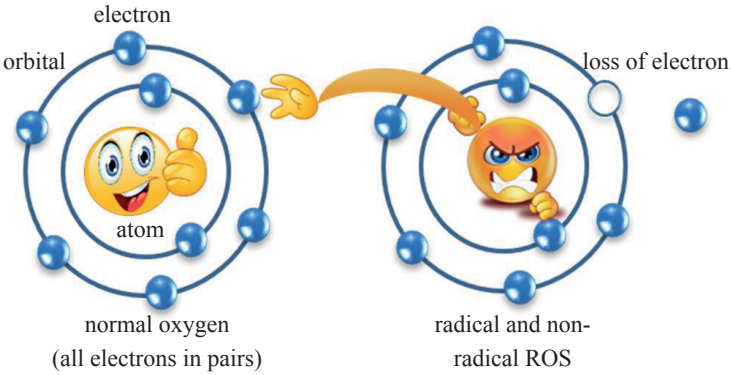


Fig. 3. Scheme of ROS formation

Reactive oxygen species (ROS) are one of the group of chemically reactive free radicals and non-radicals derived from oxygen formed as a natural by-product of the oxygen metabolism and have important roles in cell signalling and homeostasis. Since oxygen in its basic state has two unpaired electrons in outer layer,

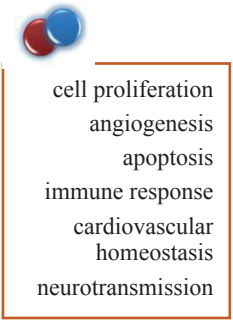


Fig. 4. Nitric oxide functions

it makes it very unstable atom, readily accepting electrons and forming various ROS. Reactive oxygen species include superoxide, hydroxy radical, alkoxyl, hydrogen peroxide, ozone and hypochlorous acid.

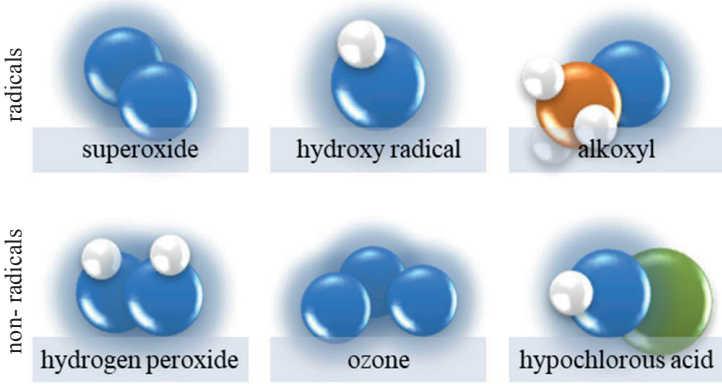


Fig. 5. ROS: radicals and non-radicals

Reactive nitrogen species (RNS) are a family of antimicrobial molecules derived from nitric oxide and superoxide catalyse with enzymatic activity of nitric oxide synthase 2 and NADPH oxidase. Nitric oxide synthase is expressed mainly in macrophages after induction by cytokines, microbial products, interferon-gamma, and lipopolysaccharide. RNS cause nitrosative stress and together with ROS damage cells and impair the functions of cellular constituents. RNS are produced in animals starting with the reaction of nitric oxide with superoxide to form peroxynitrite and they ongoing synthetized as by-products of aerobic metabolism in plants or as a response to stress.

An antioxidant is “any substance that delays, prevents or removes oxidative damage to a target molecule” (Halliwell and Gutteridge, 2007). Civilization diseases are mostly chronic or degenerative diseases which are characterized by raised levels of oxidants and/or not sufficient level antioxidants and in consequence are more receptive to oxidative stress. There are scientific evidence that antioxidant supplementation in chronic and degenerative diseases can be favourable. The antioxidant system efficacy depends on ability of cells to scavenge excess reactive species. The antioxidants may be of endogenous and exogenous origin. The endogenous anitoxidants consistent of non-enzymatic antioxidants such as glutathione (GSH), vitamins C and E and others and enzymatic antioxidants including glutathione peroxidase (GPx), superoxide dismutase (SOD) or catalase (CAT). The exogenous group of antioxidants consists of the administered micronutrients and other compounds. (Halliwell and Gutteridge, 2007). Antioxidant properties of honey *in vitro* can measure in the form of antiradical activity using various tests: DPPH - 1,1-diphenyl-2-picrylhydrazyl scavenging assay, ORAC - oxygen radical absorbance capacity assay or FRAP - ferric reducing antioxidant power assay (Gheldof *et al.*, 2002). The antioxidant activity of honey is mainly associated with the presence of phenolic compounds and flavonoids in honey. Among them the most important are included in most type of honey: quercetin, luteolin, kaempferol, alangin, and isorhamnetin and other that are typical just for few honey

varieties: ellagic acid, gallic acid, syringic acid, benzoic acid, cinnamic acid, ferulic acids, myricetin, chlorogenic acid, caffeic acid, catechin, hesperetin, coumaric acid, isoramnetin, chrysin, and galangin.

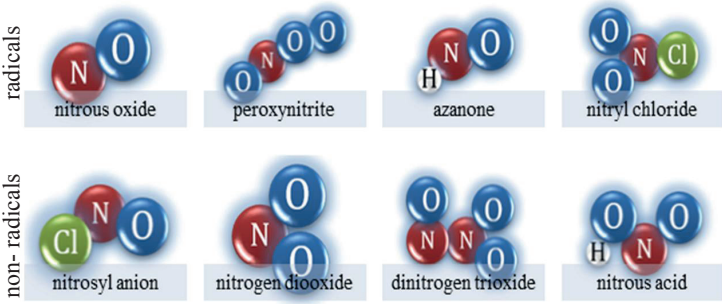


Fig. 6. RNS: radicals and non-radicals

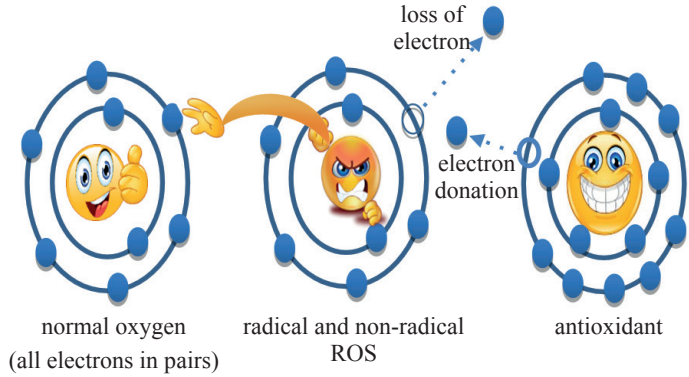


Fig. 7. RNS: radicals and non-radicals

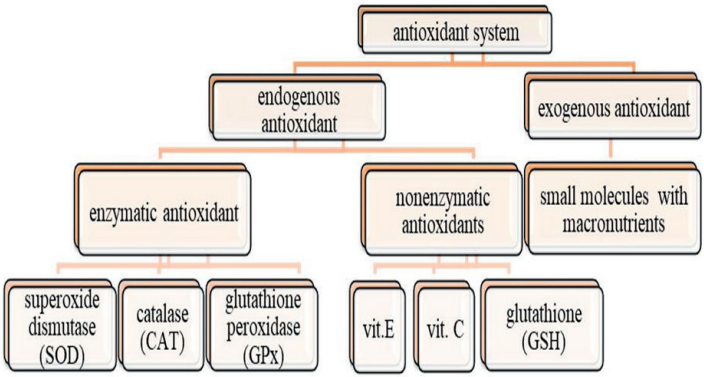
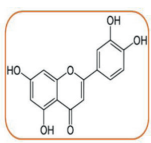


Fig. 8. Antioxidant defense system

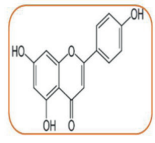
Many chronic and degenerative diseases are associated with oxidative stress and elevated levels of ROS/RNS in organism that impair cellular function. To prevent oxidative stress, the cell creates defense system comprising free radicals and other factors such as peroxidase, vit. C, vit. E, superoxide dismutase and polyphenols. Taking into consideration their activity these agents can be defined as antioxidants. Their aim is to stimulate proteins, carbohydrates, nucleic acids, and lipids to induce antioxidant response. The antioxidant capacity of honey is strong and plays the significant role in prevention of so-called civilization diseases – cancers, cardiovascular diseases, diabetes, and others. The antioxidant activity of honey depends on floral and geographical origins. Moreover, honey administered to humans in amount of 1,2g/kg of b.w. raised the activity of other antioxidants – vit. C, beta-carotene, or glutathione reductase. The detailed mechanism of honey

antioxidant activity is not well-recognised, but it is considered that antioxidative properties are associated with hydrogen donation, chelation of metallic ions, and sequestration of free radicals, superoxide radical activity, and flavonoids proper ties for hydroxyl.



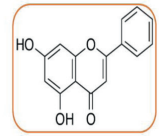
LUTEOLIN

- antihypertension, anti-inflammatory disorders, and cancer; pharmacological agent for the treatment of age-related neurode-generative disorders



APIGENIN

- strong anti-inflammatory, antioxidant, antibacterial and antiviral activities, antihypertension, suppress many human cancers by e.g. triggering cell apoptosis and autophagy



CHRYSIN

- is neuroprotective, improves cognitive decline, and is effective in neurodegenerative diseases such as Alzheimer and Parkinson diseases

Fig. 9. Structure and properties of honey flavones

Honey and Wounds Healing

Honey has a broad-spectrum antimicrobial property against proliferation of many strains of pathogens like for instance *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, E. coli*, and others. The antibacterial activity is associated with presence of some antibiotic substances in honey, including hydrogen peroxide catalyzed by glucose oxidase, osmotic effect due to high sugars content, low pH, lysozyme, flavonoids, phenolic acids, and antimicrobial peptides, especially bee defensin-1. Also, methylglyoxal and dihydroxyacetone (precursor of

methylglyoxal) have been recognised as urease enzyme inhibitors.

Catalyzing the ammonia synthesis in acidic environment, this enzyme promotes bacteria growth and consequently bacterial proliferation inhibition. There mechanism of honey activities against bacterial infection is two-directional. The first direction is inhibition of bacterial quorum sensing (QS) system to delay and limit the expression of las, MvfR, and rhl regulons, and virulence factors. The crucial is also the presence of bactericidal components, such as hydrogen peroxide, glucose, methylglyoxal etc. that demonstrates bacterial cells destroying properties. A crucial factor in antibiotic resistance of bacteria is biofilms that protect bacteria from antibiotics leading in consequence to persistent infection. Bactericidal constituents of honey can penetrate biofilms, heal severe infection, and eliminate bacteria colonies. The honey has been reported activity against biofilms of pathogenic strains such as *Staphylococcus aureus, Klebsiella pneumonia, Staphylococcus epidermidis, Pseudomonas aeruginosa, enterohemorrhagic E. coli*. Honey promotes injury treatment, acts against pathogens proliferation, prevents biofilm growth through binding strains of bacteria with fibronectin and decreases expression of Sfb1 and Sof - fibronectins binding surface proteins in the wounds (Maddocks *et al.* 2012).

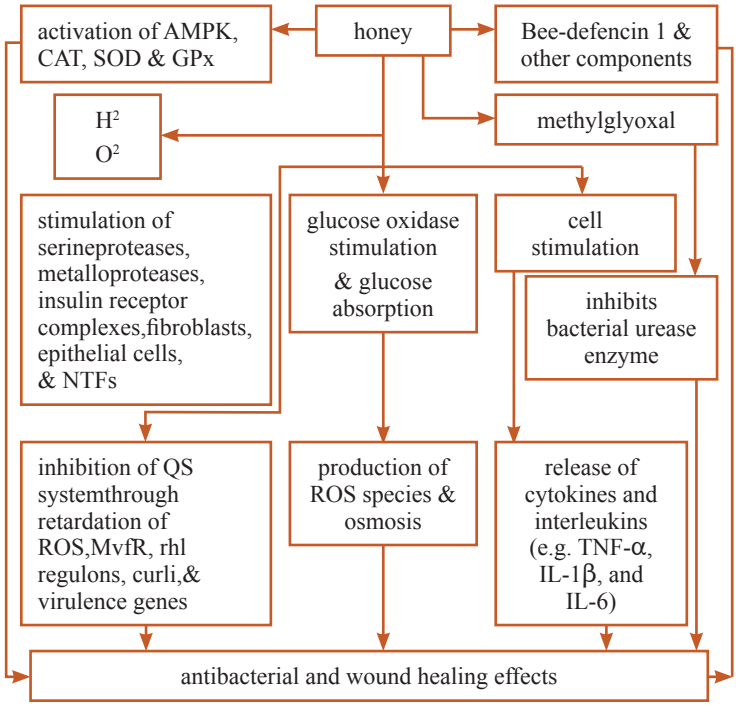


Fig. 10. Mechanism of honey wound healing (Sarfaz et al. 2018)

- AMPK - 5′adenosine monophosphate-activated protein kinase
- QS - quorum sensing
- SOD - superoxide dismutase
- GPx - glutathione peroxidase
- NTFs - nuclear transcription factors.
- TNF-α – tumournecrosis factor alpha
- IL - interleukins

Normal wound healing is a multistage process including coagulation, inflammation, cell proliferation, tissue remodeling, and replacement of damaged tissue. Many types of wounds – burn, necrotic, diabetic foot, chronic, and other wounds may be successfully healed with honey. Simulation and synthesis of defense blood cells including lymphocytes, monocytes, macrophages, and phagocytes to release interleukins and cytokines, improv-ing treatment process, are promoted by honey.

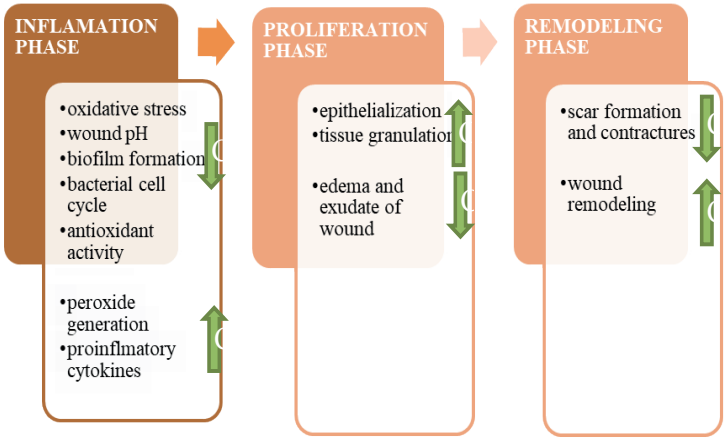


Fig. 11. Effect of honey during individual wound healing phases (Sarfraz et al. 2018)

High osmolarity thanks to high sugars content in honey also facilitates the healing process through lymph outflow. Moreover, antioxidants included in honey activate the enzyme AMPK (5′adenosine monophosphate-activated protein kinase) that reduces oxidative stress and improves the

healing process. Generally, in the wound two types of protein-digesting enzymes are present: serine proteases and matrix metalloproteinases. Usually, they are inactive. The hydrogen peroxide has the ability to deactivate their inhibitors and enables active protease bacteria digestion and easily remove the debris thanks to osmotic outflow. Additionally, to enhance wound healing hydrogen peroxide stimulates fibroblasts, epithelial cells growth, as well cell multiplication (activation of nuclear transcription factor).

Antifungal Activity

Honey shows antifungal activity against *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Microsporum gypseum, Candida albicans, Saccharomyces,* but the mechanism of this activity has not been well-recognised. Growth of fungi is inhibited by honey due to protection from formation of biofilm, destroying of already created biofilm, and initiation of changes of ecopolysaccharide structure what damage the integrity of cell membrane, reduce the cell surface in biofilm, and lead do cells' death or proliferation retardation. Studies have demonstrated that the biofilm treated with honey solution (40% w/v) effect on the thickness reduction of the exopolysaccharide layer to half. Some of honey flavonoids inhibit fungal growth, their germ-tube growth, morphology, and cells' mem-brane integrity. Hone flavonoids are recognised to reduce the percentage of cells in G0/G1 and/or G2/M phase and thus affect hyphal transition of fungi.

Antiviral Activity

Among honey constituents that exhibit antiviral inhibition through disturbing viral transcription and translation processes, the most important are vitamin C, hydrogen peroxide, flavonoids or copper. Aa a key honey compound playing an antiviral role has been identified as nitric oxide and its metabolites – nitrate and nitrite found in secretion from salivary and pharyngeal glands of honeybee. Nitric oxide has been demonstrated to be effective against both RNA and DNA viruses. It reduces viral lesion growth and prevents their replication through acting with viral polymerase, proteins of viral capsid and nucleic acid.

Anti-inflammatory Activity

Inflammation is a defense, complex process aiming to remove harmful stimuli or pathogens responsible for injury. There are two main types of inflammation: acute and chronic. The most characteristic symptoms of acute inflammation are wound redness, pain, and itching. Not treated or not adequate treatment of acute inflammation can become chronic. Chronic inflammation is supposed to induce some diseases like cancer, kidney, or liver diseases. The anti-inflammatory effect of honey has been demonstrated in many studies; however, the mechanism of this action is having not been well-recognised yet. The anti-inflammatory response begins from activation of mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) pathways what stimulate induction of several other inflammatory agents - cyclooxygenase-2

(COX-2), lipoxygenase 2, (LOX-2), C-reactive protein (CRP), interleukins and TNF-α. Reduction of edema and plasma levels of proinflammatory cytokines - IL-6, TNF-α, PGE2, NO, iNOS, and COX-2 have been reported in recent studies on honey anti-inflammatory effect. Some of honey flavonoids and phenolic acids – quercetin, galangin, and chrysin are associated with suppression of some inflammatory-promoting enzymes such as cyclooxy-genase-2 (COX-2), prostaglandins, inducible nitric oxide synthase (iNOs), and inhibition of snit-inflammatory cytokines expression. The second putative mechanism of honey anti-inflammatory effect is associated with strengthening the inflammation process ROS/RNS produced by monocytes, neutrophils, and macrophages and the ability of honey to inhibit such cells production. Also, hydrogen perox-ide promotes fibroblasts and epithelial cells growth to limit the consequences of in-flammatory response.

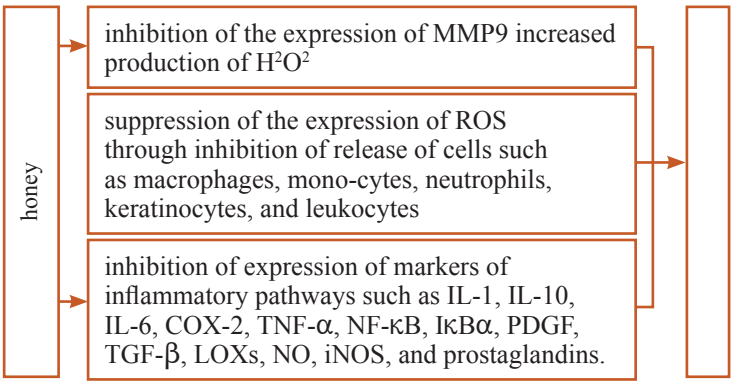


Fig. 12. Mechanism of honey anti-inflammatory activity (Sarfraz et al. 2018)

- MMP-9 - matrix metallopeptidase 9
- IL – interleukin
- COX-2 - cyclooxygenase 2
- LOXs – lipoxygenases
- TNF-α - tumour necrosis factor alpha
- PGE2 - prostaglandin E2
- NO- nitric oxide
- iNOS - inducible nitric oxide synthase
- NF-κB - nuclear factor kappa B; IκBα - inhibitor of kappa B
- PDGF - platelet-derived growth factor
- TGF-β - transforming growth factor-β

Antidiabetic Activity

The deficiency of non-functional insulin is responsible for metabolic syndrome called diabetes mellitus that is also associated with numerous abnormalities in lipid and sug-ar metabolism, finding results in ketoacidosis, hyperosmolar or hypoglycaemia. Stud-ies have demonstrated honey hypoglycemic effect when administered both orally and as inhalation with 60% water solution (w/v). Currently, the dietary meaning of carbohydrates is often expressed in terms of the glycemic index (GI). The lower GI of carbohydrates is, the lower glucose increases in blood they induce which is not insignificant for human health and diabetes occurrence. The negative correlation between GI level and fructose content has been reported.

Presence of fructose in honey is recognized to be spiritus movens of antidiabetic or hypoglycemic effect of honey. Fructose supports on the insulin-response system, what in turn results in controlled blood glucose level.

Oligosaccharide palatinose (sucrose) present in honey delays of digestion and absorption what result in reduced blood glucose level.

Fructose express also a specific hypoglycemic rolne in liver. Fructose stimulates the phosphoryla-tion enzymes activating hepatic glucose phosphorylation. The enzy-mes are inihibited and result in inhibition of glycogenolysis.

Honey treatment increased the expression of Akt and reduced the expression of IRS-1 serine phos-phorylation, NF-κB, and MAPK what improves insulin resistance and insulin contents.

Glucose captivation in cells can be increased in association with fructose and lead to decrease of food-consumption (or utilization) and hypoglicemic effect. Fructose is taken up by 2 receptors -GLUT5 and/or GLUT2, Expression of GLUT2mRNA is increased by both sugars, but GLUT5mRNA only by fructose and cause its fast absorption.

Fig. 13. Putative mechanisms of honey antidiabetic and hypoglycemic effect

Various types of honeys contain different fructose and glucose levels and hence they have different glycemic index.

Some honey such as acacia and yellow box due to high fructose content has lower IG than others. Generally, the glycemic index of honey varies from 69 to 74. Honey with low GI and they are especially recommended for people predisposed to obesity, diabetes, and with cor-onary heart disease. However, the GI concept is still an object of discussions. Currently, surplus consumption of fructose by people in developed countries, mainly in the form of high-fructose corn syrup, is suspected to be one of the main causes for overweight prob-lems due to increase in de-novo lipogenesis, which has an unfavorable effect on energy regulation and on body weight.

Antimutagenic and Anticancer Activity

Honey antimutagenic activity is strongly linked to carcinogenicity. The antimu-tagenic activity of various types of honey against Trp-p-1 and heterocyclic aromatic amines inhibition in beef steak and chicken breast mutagenicity has been reported. Due to multidirectional anticancer properties, honey affects various phases of cancer development – initiation, proliferation, and progression. Cancer cells canuncontrolledly cells proliferation and abnormal apoptosis. The standard drugs administered in anticancer therapy induce cancer cells apoptosis. Honey anticancer activity is attributed to various pathways including apoptosis initiation, cell cycle stops, effect on oxidative stress, inflammatory process improvement, estrogenic and cholesterol modulatory, ini-tiation of permeabilization of

mitochondrial outer membrane, immunomodulatory, as well prevention against angiogenesis.

Apoptosis or programmed cell death occurs in three stages: inductor, effector, and deg-radation phase. During the induction stage, the cascades of pro apoptotic signal transduction are stimulated by death-initiating signals. The cell death occurs in effector phase and mitochondrion is a key controller of this process. The last phase of apoptosis process among others includes nuclear condensation, and DNA fragmentation. Additionally, a complex of protein-breaking enzymes called caspases is activated in the cytoplasm, which finally led to cell fragmentation called apoptotic bodies and their phagocytosis. Honey has been demonstrated to exhibit anticancer effect to many types of cancer, in-cluding the most common that people are affected - breast and colon cancer. Some stud-ies on rodents have reported the antimetastatic, antiproliferative and anticancer effects of honey on breast cancer (Orsolic *et al.*, 2003). The putative mechanism of anticancer activity is anti estrogen effect, initiation of depolarization of mitochondrial membrane and apoptosis of breast cancer cells. The investigation of Tsiapara *et al.*, (2009) on three types of Greek honey – thyme, pine and fir floral origin, the authors found pine and thyme honey exhibit antagonistic activity to estrogen activity in the estradiol presence, whereas fir honey acts the opposite. Moreover, pine and thyme honey do not affect MCF-7 cell viability while fir honey promotes their viability. The researchers have drawn a

conclusion that concluded that the effect on estrogen activity was associated with high content of antioxidants in honey, especially phenolics such as kaempferol and quercetin.

Immunomodulatory Activity

Numerous chemical and biological compounds, including honey, can modi-fy the immune system. Immunomodulation may occur in natural and human-initiated forms and aims to alter an immune system in the favor of an organism's homeostasis or to induce, intensify, reduce, or prevent immune responses according to therapeutic goals – immunotherapy. An immunomodulatory response is triggered due to proliferation of blood cells initiat-ing lymphocytic and phagocytic activity and boost stimulation of immunomodulatory cytokines such as TNF-α, IL-1, IL-6, and IL-10. Honey has been demonstrated to affect the immune system in several ways. It arouses cell-mediated immunity and stimulates blood cells such as T-lymphocytes, B-lymphocytes, as well neutrophils (Morariu *et al.*, 2012). In primary and secondary immune response against thymus-dependent and -independent antigens, B-lymphocytes stimulate antibodies production. Honey stimulates higher cytokines production by monocytes and provides glucose for synthesis of hydrogen peroxide, stimulating the immune system. It is also the substrate to glycolysis process providing energy in macrophages and activates their immunomodulatory poten-tial. The putative immunomodulatory mechanism short fatty acids (SCFA) formation after honey ingestion. It has been proved

that SCFA have immunomodulatory properties and honey stimulates the immune system through fermentable sugars e.g. nigerose. Im-munomodulatory activities also have non-sugar honey constituents such as antioxidants.

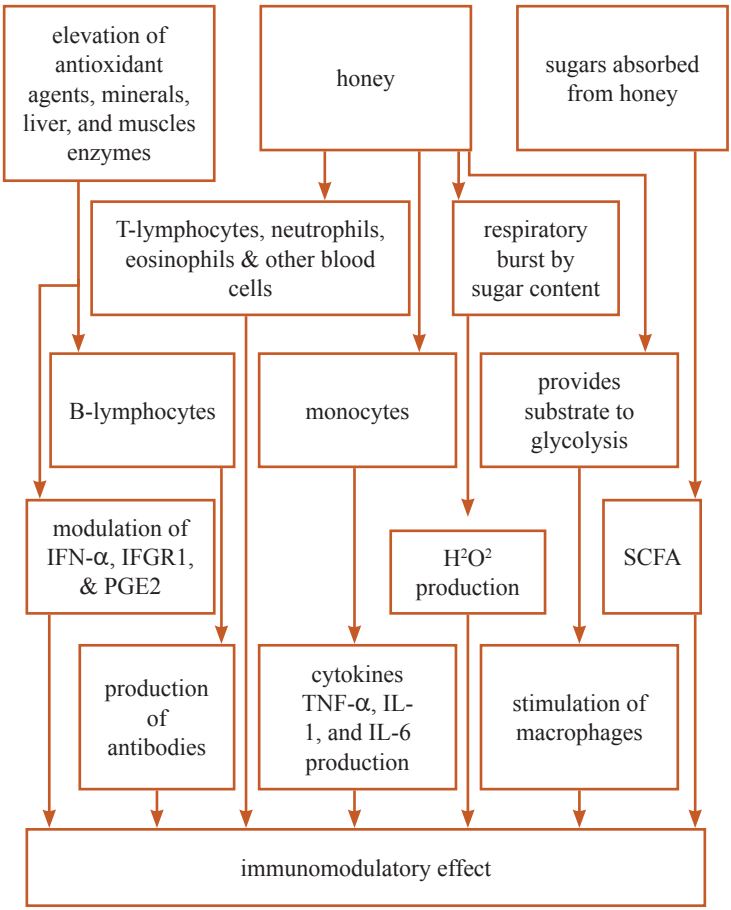


Fig. 14. Immunomodulatory mechanism of honey (Sarfraz et al. 2018)

Honey, administered to healthy humans in amount 1.2 g/kg body weight, was found to increase the antioxidant agents (vitamin C and β-carotene), monocytes, lymphocytes, eosinophils, serum iron and copper, glutathione reductase, and trace elements (Zn and Mg). The decrease in immunoglobulin E, ferritin, and liver and muscle enzymes, aspar-tate transaminase, alanine transaminase, lactate dehydrogenase, creatinine kinase, and fasting blood sugars has been reported (Al-Waili 2003). Honey contains probiotic bacte-ria that also contributes to immunomodulatory honey properties. They protect the immune system and favorably affect immunoglobulins concentration and rate of recurrence of interferon and immune phagocytic (Jassawala 2007). It is supposed to inhibit prosta-glandins synthesis. Immunomodulatory activities can be reinstated by the treatment with inhibitors of prostaglandin or by dropping systemic PGE2 levels. It has been reported that honey demonstrates inhibitory effects on PGE2 in carrageenan-induced acute paw edema in rats (Hussein *et al.* 2012).

Honey and Cardiovascular Diseases

Numerous cardiovascular risk factors such as blood glucose level, cholesterol, C-reactive proteins (CRP), and body weight are regulated by honey. Two main sugars in-cluded in honey – glucose and fructose, and some minerals, especially zinc and copper have the potential to decrease the cardiac risks. Also due to its anti-inflammatory prop-erties, honey prevents cardiovascular diseases that are associated with chronic low-

grade inflammation. Studies carried out on healthy and cardiac patients by Yaghoobi *et al.* (2008) demonstrated that honey administered in amount of 70 g by 30 days decreased LDL (low-density lipoprotein), high-density lipoprotein cholesterol (HDL-C), triacylglycerol, body fat, glucose, and cholesterol level in blood. Moreover, lowered CRP concentration was stated as what stimulates synthesis of nitric oxide. Nitric acid positively affects vascular tone and blood pressure, preventing platelet aggrega-tion, proliferation of smooth muscle cells and adhesion of leukocytes. It has been demonstrated that NO is an important agent of blood vessels vasodilation and regulates extracellular fluid homeostasis by kidneys, which is also crucial for blood pressure and blood flow control (Naseem 2005). Nitric acid is also responsible for phosphorylation of several proteins that causes smooth muscle relaxation. Moreover, honey contains flavo-noids that reduce the risk of cardiovascular diseases through diminishing the oxidative stress and improvement of nitric oxide bioavailability. Rutin included in honey raises expressions of the eNOS gene and thus, stimulates nitric oxide synthesis. Additionally, nar-ingin has been reported to inhibit hypercholesterolemia while quercetin and catechin prevent aortic atherosclerotic lesions and atherogenic modification of LDL (Afroz *et al.* 2016). Honey reduces concentration of aspartate transaminase, alanine transami-nase, glutathione peroxidase and reductase, superoxide dismutase, lactate dehydrogen-ase, triglycerides, total cholesterol, and lipid peroxidation products in blood. The precise mechanisms of cardiovascular action of honey have not been well-recognized yet.

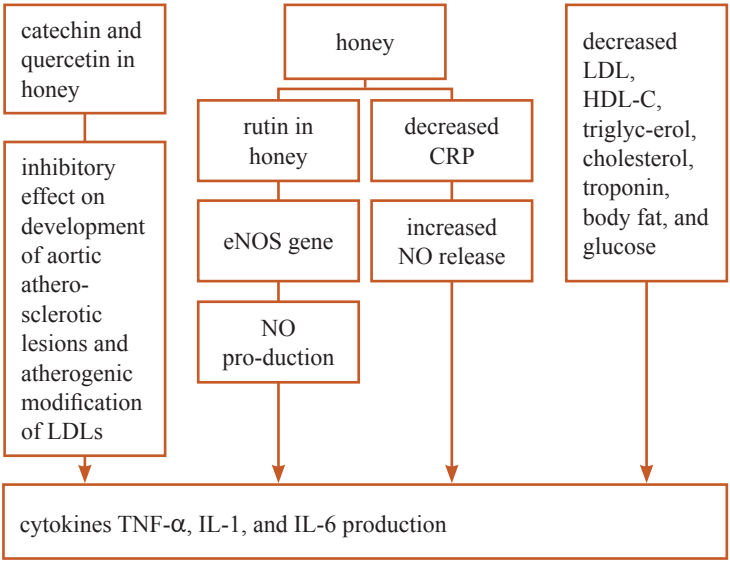


Fig. 15. Honey effects in prevention of cardiovascular diseases (Sarfraz et al. 2018)

eNOS - endothelial nitric oxide synthase
LDL – low density lipoprotein
HDL-C - high-density lipoprotein cholesterol
CRP - C-reactive proteins

Prebiotics Properties of Honey

Honey contains around 0, 75% of fructo-oligosaccharides, thus may demonstrate prebiotic properties. Fructooligosaccharides, including inulin are non-digestible oligosaccharides that promote the proliferation of beneficial colon bacteria such as lactoba-cilli and bifidobacteria. These non-digestible carbohydrates are fermented by beneficial

bacteria to SCFA. Prebiotics administered in diet affect pH decrease, lowered fat absorp-tion, ammonia production and enhancement of immune system. The fructooligosaccha-rides included in honey protect Bifidobacterium spp from detrimental effects of bile salts (Perrin *et al.* 2001). The prebiotic effect of honey has been observed in several monoflo-ral honeys: sourwood, alfalfa and sage honey, honeydew honey, chestnut and acacia honey, clover, and eucalyptus, with strength of activity highly dependent on its floral origin.

Honey and Dermatology

Honey is widely used in remedies in skin care and treatment. It has moisturizing, emollient, anti-pimple, and anti-wrinkle properties. Honey is also used in many cosmet-ic products as a binder. In traditional Indian and Chinese medicine, honey is recom-mended as a remedy for skin discoloration, freckles, spots, and scars and improves the general appearance of the skin (Oumeish 1999, Ahmad *et al.* 2008). Honey, due to the antibacterial effect, can be used in treatment of vaginal candidiasis, superficial mycoses, athlete's foot, and ringworm (Molan 1992). The antimicrobial activity of honey is mainly dependent on hydrogen peroxide and methylglyoxal content. Honey is also effective in the treatment of dandruff, tinea, hemorrhoids, and psoriasis. (Burlando and Cornara 2013). Three-month application of honey to the vagina and cervix in women with precancer-ous lesions of the uterine cervix found the effect in 95% patients with normal pap smears. Seven-day administration of honey alone or with clotrimazole

removed all symptoms and signs of vulvovaginitis. It has been also reported that honey applied on the skin can decrease the severity of rosacea. However, prolonged treatment is ineffective due to many side effects (van Zuuren *et al.* 2011).

The Supposed Neuroprotective Mechanism of Honey Polyphenols

Through an inflammatory, apoptotic, or necrotic response caused by generation of reactive oxygen species (ROS) that lead to oxidative stress and finally neuronal cell death. Oxidative stress can be countered by Honey (H) and its polyphenol constituents (HP) limiting the formation of ROS/RNS as well as by strengthening the cellular antioxidant defense system. Honey and some of its polyphenols such as catechin, ferulic acid, and pigenin prevent neuronal cell death by lessening neuroinflammation and apoptosis. How-ever, the neuroinflammatory responses overlap with apoptosis, and the role of honey in necrotic cell death remains unclear.

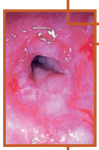
Honey and Gastrointestinal Tract Health

Due to its antimicrobial and anti-inflammatory properties as well ability to stimulate tissue repair natural honey is also effective in radiation mucositis. The study car-ried out by Biswal *et al.* (2003) on 40 patients has demonstrated that 20 ml of natural honey given to patients is 15 min. before and after, as well 6 h post radiation therapy significantly reduced symptomatic mucositis. Honey affects oral cavity and teeth health, preventing pathogenic bacteria colonization and

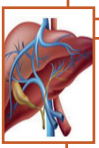
removing necrotic tissue in any injuries. Moreover, it promotes new tissue development through stimulation of fibroblast and epithelial cell proliferation as well as the angiogenesis process.

There are also some reports on honey activity against living in the stomach bacteria Helicobacter pylori that is responsible for many cases of dyspepsia. Study carried out by Al Somal *et al.* (1994) demonstrated sensitivity of Helicobacter pylori isolated from gas-tric ulcers. They reported that bacteria were sensitive to 20% (v/v) solution of manuka honey in an agar well diffusion assay but none of the tested samples have no exhibited sensitivity to 40% (v/v) solution of honey which main antimicrobial agent was hydro-gen peroxide.

Honey is a potent inhibitor of Helicobacter pylori – bacteria that is responsible for peptic ulcers and gastritis.



Due to the lower surface tension, high viscosity as well density honey coats the mucus membrane and can be used in treatment of reflux oesophagus and heartburn.



It has been reported a positive effect of honey on hepatitis – in patients after ingestion of clover and rape honey was stated decrease in alanine aminotransferase activity (by 9 to 13 times) and of bilirubin production by 2.1 to 2.6 time.

Fig.16. Some treatments of honey

The antimicrobial activity against Helicobacter pylori that are one of the agents causing gastric ulcers and the

presence of high number of flavonoids in honey are believed to have the value of pharmacological activities including preventing the for-mation of gastric ulcers via its antisecretory and antioxidant mechanisms. Moreover, the lower surface tension, high viscosity as well density of honey make it coats the mu-cus membrane and can be used in treatment of reflux esophagus and heartburn. Honey is believed to protect the liver from toxic substances. It has been reported a positive effect of honey on hepatitis – in patients after ingestion of clover and rape honey was stated decrease in alanine aminotransferase activity (by 9 to 13 times) and of bilirubin production by 2.1 to 2.6 time. Moreover, honey is reported to increase glycogen level having the significant importance in liver proper activity.

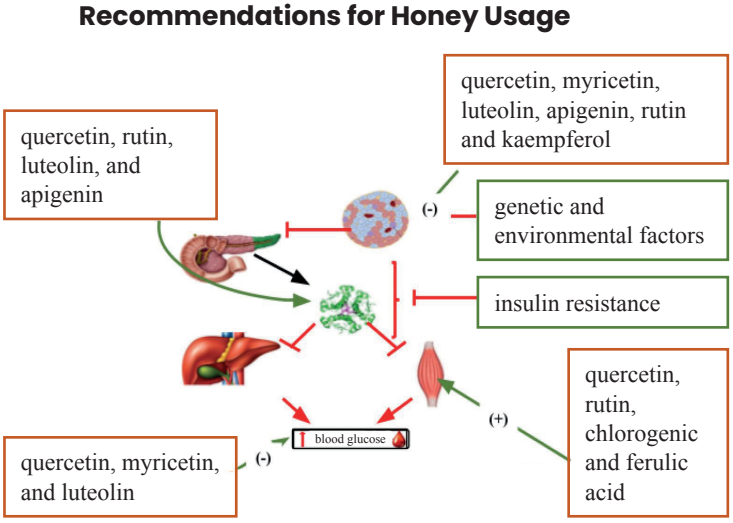
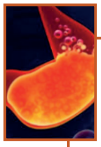


Fig. 17. Effect of honey antioxidants on pancreas and liver metabolism

The health enhancing effects in human adults, described in this report were mostly achieved after ingestion of 50 to 80 g of honey per day. Practitioner apitherapists rec-ommend ingestion for 1-1.5 month. The health effects of honey which are reported for intakes of following amounts of honey generally in adults or infants at the level from 0.8 g to 1.2 g honey per kg human body weight.

Most Popular Types of Honey and Their Properties

Currently, the market is full of a large variety of honey. There are around 300 types of honey. Taking into consideration preferences, consumers are led when choos-ing type of honey with taste, flavor, and color. Different types of honey are categorized by the flower source but even if honey was delivered from the same flower source in the same location, its taste could be different because even such factors like rainfall, and tem-perature may affect its taste and composition. However, the overall tendency is that lighter colored honey is milder in taste than the darker ones. Also, the floral source decides about honey chemical composition, type and quantities of biologically active substance and finally about therapeutic properties and recommendations for use. Below few the most popular types of honey and their properties are described



to decrease gastric juice acidity the author recommends the intake of warm honey solution 40 to 60 minutes before eating

to influence favourably cough and sleep of children - 1 to 2 tablespoons

to improve function of the gall bladder is improved by the ingestionof cool solution of 100 ml 50 % honey

generally for children - ½ tsp - 2-5 year olds, 1 tsp -6 to 11 year-olds and 2 tsp for 12 to18 year-old

Fig. 18. Use of honey and its solutions in the treatment of some diseases

Risk Related to Honey Usage

Regardless of its usage as food or remedy, honey may be contaminated by pesticides, an-tibiotics, heavy metals, and other toxic compounds that may be harmful for human health, producing unexpected consequences. In addition to those chemical compounds, honey may also be contaminated with pathogens, particularly Clostridium botulinum and its spores. The consump-tion of honey or its derivatives is dangerous for infants, the elderly, and immun-ocompromised persons. For

this reason, honey used for therapeutic purposes should be sterilized using gamma irradiation. There could also be some adverse effects or disadvantages in external usage of honey in treatment: Honey should be evaluated for its toxicological effects based on plants and or nectar source. Though not all, intoxication by honey may be expected. Grayanotoxins are a group of closely related neurotoxins found in rhododendron plants in coun-tries such as China, Tibet, Türkiye, Nepal, Myanmar, Japan, New Guinea, Philippines, Indonesia, and North America. Honey from that plant is toxic, especially this collected in spring, and it's called *mad honey*. Grayanotoxins cause intoxication which may include weakness, dizziness, excessive perspiration, hypersalivation, nausea, and vomiting or even to heart problems. Some of the plants used by bees contain poisonous substances such as diterpenoids and pyrazolidine. It was found that honey in Australia contains natural toxins -pyrrolizidine alkaloids (PAs) that exceed international safety levels. The toxins are known to cause liver damage in humans and are believed to cause cancer when consumed in high doses. The pyrrolizidine alkaloids are produced by around 600 types of pasture plants in Australia (Bogdanov 2008).Toxic honey may also result when bees gather honeydew produced by vine hopper insect (*Scoly-popa australis*) feeding on tu tu bushes (*Coriaria arborea*). The toxin called tutin is introduced to honey then. Both plants and insects are found in New Zealand. Tutin poisoning symptoms are among others vomiting, delirium, dizziness, lethargy,

coma, and convulsions. To prevent tutin poisoning, people should avoid eating honey from feral hives in threatened areas.

Fig. 19. Regions where honey with toxic substances have been reported

Methods for Harvesting Honey

Bees should be kept away from honeycombs during honey harvest. The methods used for this purpose are as follows:

- **Shaking and brushing:** After shaking and brushing, the frames are picked up and taken to the harvest room.
- **Beekeeper method:** Beekeepers are placed in the middle of the inner cover of the hive and on the honeycombs to be harvested. Then the roof of the hive is opened a little and the inside is smoked.
- **Filtering and resting the honey:** The honeycombs and containers to be filtered are taken to the extraction

room. All frames are removed and the glaze on the honeycombs is scraped off with a glaze comb or glaze knife.

- The honeycombs, whose secrets have been removed, are placed in the honey extraction machine. These machines have a mechanism based on centrifugal. There are different types of these percolators such as electrically operated and manually operated. At the end of the process, some honey residue remains in the drained combs. Those combs should be given to stronger hives, cleaned, and repaired, and distributed to other hives the next day. Honey obtained from the filter machine is not clean.
- It contains parts, larvae, dead bees, and pollen grains. A zero numbered wire sieve is used to eliminate impurities in honey. After filtering, the honey is transferred to resting containers. It protects the hive. It ensures the hygiene of the hive by preventing the development of various spores and the like.

Photo 6. Methods used to harvest honey.

Storage of Honey and Thawing Frozen Honey

Crystallization, which is a natural change, can be controlled by appropriate storage, heating, or filtering. Another way to prevent honey from crystallizing is to keep it at 0 °C for at least 5 weeks and then store it at 14 °C. The healthiest packaging for honey is a glass jar. The containers in which honey is stored, humidity, heat and light in the environment affect crystallization. Apart from this, in filtered honey, air bubbles in the honey cause crystallization in pollen, garbage, dust, wax, propolis and other foreign substances. Crystallized packaged honey becomes liquid again if it is kept in water at 45 °C in an air-dry cabinet or in a boiler with adjustable

temperature, whose temperature is kept at 45 °C. While doing this process, the heating process should be finished as soon as the thawing is completed in order not to lose some of the beneficial properties of the honey.

Photo 7. Honey Processing Plants

Storage Conditions

· Firmness

One of the most important storage conditions is a tightly closed glass container.

· Lighting

Store honey in a dark place. You should not leave jars in the apartment: on the kitchen table or on the windowsill. When direct sunlight hits a bee product, especially in a transparent container, its medicinal and nutritional value is significantly reduced. For a product stored in a dark container, it is also undesirable to stay in a strongly lit place for a long time, especially because of the danger of overheating. Diffused light

entering the cabinet through a glass insert also has a negative effect on the antimicrobial qualities of the product.

· Humidity

Honey can absorb moisture from the environment. Therefore, to best preserve the beneficial properties of the product, they choose a dry place with optimal humidity of about 60%. Its ability to be hygroscopic, especially in rooms with high humidity, causes the consistency of the product to become liquid and deteriorate. The room should be well ventilated to avoid mold, this will also determine how much honey is stored.

· Smell

Honey easily absorbs odors, so it is not recommended to store it near spices, garlic or onions, pickles, and other smelly substances such as gasoline or paint. You should also not place jars next to bulk products such as flour - due to the sticky consistency of honey, flour particles can settle on its surface, causing fermentation. The smell of tobacco or smoke may enter the composition of the nectar.

· Heat

Healthy sweetness is stored only in a cool place. At temperatures above + 20C °, the beekeeping product loses its healing properties, turning into an ordinary sweet mass, so it should not be stored in cabinets near the stove or heated

radiator. The ideal container for storing honey is a dark glass jar with a sealed lid. The presence of a rubberized or plastic seal on the lid is allowed for a tighter closure. Consider other suitable storage container materials.

- **Wood**

Suitable barrels made of alder, birch, linden, or beech, with a moisture content of not more than 16%, impregnated with wax from the inside. Pots made of coniferous wood are not used, as such dishes give off tar and emit odors. Oak barrels dry out over time, lose their firmness, and the honey inside turns dark.

- **Clay**

For storage, use clay, ceramic, or porcelain containers with tight closures. For long-term storage, the dishes can be sealed with wax at the junction with the lid. The inside of ceramic pots should be glazed.

Clay has a porous structure that allows it to maintain a suitable temperature. But you should also consider the property of the material to absorb odors, therefore, before using a clay pot from scented products, it should be washed without the use of detergents, especially with the content of chemical elements in the composition and ignited in the oven. Pottery, ceramics, and porcelain dishes have one drawback - increased brittleness, especially with temperature changes.

· Plastic

Plastic containers for storage or transportation may be used along with those marked "for food." Honey can interact with and absorb the chemical elements of non-food plastic. Therefore, if sweet amber purchased in a plastic bottle raises doubts about its suitability, then it should be poured into a more suitable container at home. Even food grade plastic is still not recommended for long-term storage of the product.

Other Materials

- It is allowed to store honey in stainless steel and aluminum containers. However, it is dangerous to leave the product in an iron, copper, or galvanized container for a long time. Honey in such containers interacts with oxidized metal and forms chemical compounds that are harmful to health. For the same reason, it is not recommended to use an iron spoon for a set of desserts or leave them in bulk.
- Important! When storing honey in an enamel container, the presence of chips or other damage is not allowed.
- Make sure the storage containers and lid are clean and dry. You cannot pour a new portion of nectar into a jar that has not been cleared of previous residues. The residues left behind meet the fresh product, causing it to ferment. It is best to use a permanent, tested container, but not jars and other strong-

smelling products to avoid odors in fresh honey.

- The residence time of medicinal substances and trace elements in the product composition depends on the storage location.
- What is the best storage space for a refrigerator or pantry? Honey can be stored in the refrigerator, in a compartment with a temperature of + 5C °, for example, on the door. However, changes in humidity in the refrigerator, intermittent lighting, and various strong odors from other products can make storage difficult. True, in conditions of high room temperature, without the possibility of adjustment, the refrigerator becomes the only place and way of proper storage of honey, if containers with closed lids are used. Sweet amber is allowed to be stored in cool loggias in cabinets. Storage locations should not be changed frequently. Important! Temperature changes negatively affect the quality, color, and smell of honey. By moving jars from the refrigerator to rooms with room temperature, you should not change storage places frequently to avoid the appearance of condensation.

Is it possible to freeze honey jars?

Honey can be frozen in a freezer at a temperature not lower than -20C °. However, this method does not affect the shelf life and makes it difficult to remove the product from

the container after it is at a lower temperature. Therefore, for such storage, you should choose small containers, given that the mass of liquid will increase when freezing. For this reason, the bee product is not poured into the container to the brim, leaving space at the top. Than the honey at room temperature without removing the lid.

Check Yourself

1. **Average moisture content in honey is:**
   a) ca. 8%
   b) ca. 18%
   c) ca. 38%
   d) ca. 58%

2. **The most abundant sugars in honey are:**
   a) fructose and glucose
   b) glucose and sucrose
   c) sucrose and maltose
   d) fructose and lactose

3. **Honey is the main source of:**
   a) calcium
   b) sodium
   c) potassium
   d) magnesium

4. Choose the false sentence:

a) Glucose oxidase is synthetized in the hypopharyngeal glands of honeybee workers.
b) Glucose oxidase is deposited in honey.
c) Glucose oxidase is an antimicrobial barrier reducing at the surface of the honey atmospheric O2 to hydrogen peroxide.
d) Glucose oxidase activity decreases clearly in honey diluted with water.

5. Choose the false sentence. Mad honey….:

a) is made of canola plant.
b) contain grayanotoxins
c) is made of rhododendron plants.
d) is especially toxic when collected in spring.

6. Unsterilized using gamma irradiation honey can be dangerous, especially for infant due to possible:

a) *Salmonella typi* contamination
b) *Eschericha coli* contamination
c) *Clostridium botulinum* contamination
d) *Lactobacillus spp*. contamination

7. Honey with the highest antimicrobial activity is:

a) honey made of *Brassica napus*
b) honey made of *Leptospermum scoparium*
c) honey made of *Medicago sativa*
d) honey made of *Trifolium repens*

8. Honey prevents from dental caries activity thanks to antimicrobial activity against:

a) Streptococcusmutans

b) Enterococus faecium

c) Lactobacllus buchneri

d) Shigella

9. The putative mechanisms neuroprotective of honey results from:

a) honey antimicrobial activity and inhibition of *Streptococcus mutans* proliferation

b) high sugars content in honey

c) there are no evidenced for such honey activity.

d) honey antioxidants activity and limiting the formation of ROS/RNS

10. The properties of honey that make it effective against bacterial growth are:

a) hydrogen peroxide

b) high sugar content

c) gluconic acid

d) all answers are correct

Answers: 1b, 2a, 3c, 4d, 5a, 6c, 7b, 8a, 9d, 10d

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Propolis

Prof. Dr. Kemal ÇELİK
Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

A large part of the plants protects their leaves, flowers, and fruits against antimicrobial decay with waterproof and heat-insulating resinous substances. Honeybees collect these resinous substances from cracks, buds, and leaves in the trunks of trees. The sticky substance mixed with beeswax is used for various purposes in the hive, by chewing the bees, adding, and digesting the digestive enzymes and partly digesting them. In other words, propolis honey bees; collected from live plants, mixed with beeswax and larvae before laying eggs in the eyes and the polishing of the inside of the hive is an adhesive, dark-colored substance used to close.

History of Propolis

The term propolis is derived from Greek and the pro means “defense” and the police means “city”. From there, it was possible to reach a meaning such as the defense of the city or the hive. The discovery of propolis dates to the years before Christ. The well-known Greek philosopher Aristotle

wanted to study the bees' work by using a transparent hive, but the transparency of the hive was darkly covered with waxy substances. This dark-colored substance is estimated to be propolis. The positive effects of propolis on human beings have been known since ancient times and their use among the people is based on ancient times. First BC. In 79-23 years, Pliny the Elder, a large school in Rome, described the pain-reducing, wound-healing activities of propolis. Propolis was also known by the Egyptians in ancient times and was used for the treatment of some diseases and for the embalming of the dead. The Greeks and Romans used propolis for centuries to treat skin abscesses. Hippocrates (460-377 BC) stated that propolis is used in the treatment of skin diseases, ulcers and digestive cystermia. In Africa, propolis has long been used as a medicine. Medical records of propolis used for oral, throat infections and dental health have been described in European records of the 12th century. Another use of propolis based on ancient times is the use of varnish. In Italy, in the 17th year, Stradivari used propolis in the polishing of stringed instruments. The most important and well-known feature of propolis that has come from past times to the present is its effect against microorganisms. Propolis is used by people today because of its properties. In the century we live, this valuable bee product has numerous useful biological activities such as anti-inflammatory, anti-inflammatory, anti-ulcer, local anesthetic, antitumor, immunosuppressive as well as antibacterial, antifungal, and antiviral properties; and its use

in medicine, apitherapy, health food and biocosmetic fields. In recent years, propolis has gained importance as a health drink. It is also widely used in foods, and it is thought that it improves human health and removes heart diseases. These properties of propolis have attracted the attention of scientists since the end of the 60s. Over the last 40 years, many studies have been published on biological use, pharmacological and therapeutic uses of chemical use. The first comprehensive research was published by Ghisalberti in 1979. Nowadays, much work has been done on the chemistry and biological activity of propolis. However, there are various difficulties associated with the administration of propolis. The main reason for this problem is that the chemical composition of propolis varies considerably depending on the vegetation and season of the region. Because different plants in different ecosystems and the secretions of these plants can be a source of propolis. For these reasons, the standardization of propolis has not yet been fully achieved. In today's world, the increasing number of events that threaten human health such as stress and environmental pollution have made the negative impact of environmental conditions more frequent. Despite these negative effects of living conditions, propolis is being studied for various purposes in many countries due to its properties such as increasing body resistance, acting as an antibiotic and most importantly being a natural product. In many countries abroad, a variety of commercial products are produced using propolis. According to the literature, propolis was first used commercially in the 1950s. 1984 records of

propolis; It includes the export of 55 tons of propolis from China, smaller quantities from Argentina, Canada, Chile, and Uruguay, and at least 11 other countries with unknown quantities.

Honey, which has an important place in the balanced and healthy nutrition of people, as well as other bee products such as pollen, royal jelly, propolis and bee venom, are now used for many purposes. In recent years, the content, and effects of propolis, which is another important bee product as much as honey, were determined and the usage area was increased. The effect of propolis against microorganisms, which is used as a natural antibiotic by discovery in ancient times, is the main character and has been used by people since ancient times due to its drug properties. The pharmacological properties of propolis have been described by the Greek and Roman physicists Aristotle, Dioscoroides, Pliny and Galen. According to this definition, propolis can be used as an antiseptic in the treatment of wounds and oral infection. These properties of propolis were used in Europe and Arabia in the Middle Ages. Inca's used propolis as antipyretic. Propolis 17th century. In London, it is listed as the official drug and again in these years due to its antibacterial activity has gained importance in Europe. Propolis has gained importance with its excellent natural product characteristics which was discovered in the present century to contain 22 components that should be taken for human health. Propolis is a very interesting bee product for further research. In many countries, propolis

contains some questions that have not yet been answered, although they are used against various medical problems. This limits the use of propolis in modern medicine. Propolis's different fields of use have attracted the attention of scientists and various research has been started. Due to this interest, the commercial importance of propolis has also increased. Propolis is a natural product with great potential in veterinary and human health. On the other hand, unlike the products obtained from medicinal plants, their contents vary greatly. The chemical contents of the propolis samples collected in different countries vary widely. This diversity poses a serious problem for the medical use and quality control of propolis. The biggest problem faced is that the origin of propolis varies from region to region. The unknown origin of propolis causes serious problems in standardization.

Today there are various uses of propolis, pure or aloe gel with pollen, as extract (hydroalcoholic or glycolic), as mouth spray (melissa, sage and / or mixed with rosemary), throat lozenges, creams and powdered, mouthwash is produced as and after the wax is removed. Despite the numerous effects of propolis, most of the reports are based on preliminary studies. Most studies are conducted in Eastern European countries. Applied studies and research are mainly conducted in China. But the provision of information is difficult because of the language barrier. More detailed studies, especially intestines, skin and dental practices will help to determine the possible benefits of propolis in medical use. Although there are no

official records of Propolis production, it is estimated that approximately 200 tons of propolis were sold in the world market in 1984. Among the countries producing the most propolis are China, Brazil, America, Australia, and Uruguay. Japan is leading the processing and consumption of propolis. Lack of synthetic production of propolis, patent and standard problem, beekeeping of uneducated people; Honey, pollen and bee milk do not have a marketing network at the level and the source of income as a source of satisfaction of beekeepers and private firms to prevent the spread of propolis production is prevented. In Turkey, propolis microscopic and chemical analyzes made by various researchers and Turkey of plant sources of propolis, usually *Castanea sativa* and *Populus spp*. It has been reported to be. However, there are no detailed studies covering a region. Thus, Türkiye has not established standards covering propolis. A study of the chemical composition of propolis Türkiye Sorkin *et al.* (2001). In this study, samples from different regions of Türkiye (Bursa, Erzurum-Aşkale, Trabzon and Gumushane-Sogutlugil-Cascade) were collected and chemically analyzed by GC-MS. According to the results of this study, similar chemical content was observed in propolis samples taken from Trabzon and Gümüşhane regions and Erzurum sample showed a different structure. In the samples collected from the Bursa region, flavonoids, flavones and ketones were found to be quite rich. Eagle *et al.* (2002), Turkey's studied antimicrobial activity of propolis samples collected from the Ankara-Kazan and Marmaris. Prepared 4

different ethanol extracts (using 30%, 50%, 70% and 96% ethanol) from the samples of propolis and examined the effects of these extracts on 7 Gram (+), 4 Gram (-) and a fungal culture. They stated that the samples taken from Ankara-Kazan showed stronger antimicrobial activity than the samples of Marmaris and that the chemical content of Ankara-Kazan propolis was like that of Populus species. They explained that the observed activity is mostly caused by caffeic acid and its esters. Sorkun *et al.* (1996) studied the photoinhibition effect of propolis. Türkiye from different regions (Çankırı, Aksaray, Milas-Pen, Gümüşhane Kaletaş) in different concentrations in samples collected ethanol extracts (EEP) prepared. According to the findings, propolis solutions significantly inhibited the percentage of germination depending on the concentration. It was observed that the seed in which EEP was applied inhibited the mitotic compartment in the stem cells. Propolises collected from Milas-Kalemli and Çankırı regions significantly inhibited mitosis and most inhibition was observed in Çankırı propolis.

Propolis Characteristics

Physical Properties of Propolis

The colour of propolis ranges from yellow to dark brown depending on the origin of the resins. Propolis, the color of yellow to dark brown, sometimes green. Below figure shows the raw propolis in brown collected from the hive. Propolis color varies according to the region and season. For example, countries with temperate climates have distinct brown, while

in the tropical climate and in Australia, propolis is black. The Finland propolis is orange, and the Cuban propolis is dark violet. But even transparent propolis has been reported by Coggshall and Morse (1984). It is natural to observe differences in the color of the propolis due to changes in botanical origin.

Photo 8. Raw propolis

Propolis is a mixture of various amounts of beeswax and resins collected by the honeybee from plants, particularly from flowers and leaf buds. Since it is difficult to observe bees on their foraging trips the exact sources of the resins are usually not known. Bees have been observed scraping the protective resins of flower and leaf buds with their mandibles and then carrying them to the hive like pollen pellets on their hind legs. It can be assumed that in the process of collecting and modeling the resins, they are mixed with some saliva and other secretions of the bees as well as with wax. These resins are used by worker bees to line the inside of nest cavities and all brood combs, to repair combs, to seal small cracks in the hive, to reduce the size of hive entrances. They also use them to seal off inside the hive any dead animals or insects which are too large to be carried out and, perhaps most important of all, to

mix small quantities of propolis with wax to seal brood cells. These uses are significant because they take advantage of the antibacterial and antifungal effects of propolis in protecting the colony against diseases. Propolis has been shown to kill the bee's most ardent bacterial foe, *Bacillus* larvae - the cause of American Foul Brood (Mlagan and Sulimanovic, 1982; Meresta and Meresta, 1988). The use of propolis thus reduces the chance of infection in the developing brood and the growth of decomposing bacteria in dead animal tissue.

At temperatures of 25º to 45 ºC propolis is a soft, pliable, and very sticky substance. At less than 15ºC, and particularly when frozen or at near freezing, it becomes hard and brittle. It will remain brittle after such treatment even at higher temperatures. Above 45 ºC it will become increasingly sticky and gummy. Typically, propolis will become liquid at 60 to 70ºC, but for some samples the melting point may be as high as 100ºC. The most common solvents used for commercial extraction are ethanol (ethyl alcohol) ether, glycol, and water. For chemical analysis a large variety of solvents may be used to extract the various fractions. Many of the bactericidal components are soluble in water or alcohol.

Chemical Properties of Propolis

In general, the chemical content of propolis is very complex and varies depending on the flora of the area it is collected. Depending on the species and density of plants grown in different ecosystems, the chemical content of

propolis obtained from these regions varies. The content of propolis differs depending on the flora, climatic conditions, the amount of resin in the bud, the time of collection, wax, pollen and the content of the substance secreted by the bee in addition to the local flora. Bee species and bee breed are among the factors affecting the content of propolis.

Constituents of propolis	% rate
Herbal wax	30
Essential Oils	10
Organic Compounds and Mineral Substances	5
Pollen	5
Resin and Gum Ingredients	50

Table 7. General composition of propolis

In one recent analysis of propolis from England, 150 compounds were identified in only one sample (Greenaway, *et al.*, 1990), but in total more than 180 have been isolated so far. It appears that with every new analysis, new compounds are found. Propolis resins are collected from a large variety of trees and shrubs. Each region and colony seem to have its own preferred resin sources, which results in the large variation of color, odor, and composition. Comparisons with tree resins in Europe suggest that, wherever Populus species are present, honeybees preferably collect the resins from leaf buds of these trees. A Cuban study suggests that the plant resins collected are at least partially metabolized by bees (Cuellar *et al.*, 1990). The presence of sugars (Greenaway *et al.*, 1987) also suggests some metabolization by bees, i.e. because of adding saliva

during both scraping and chewing. A list of the major classes of chemicals occurring in propolis is given below with references to some recent reviews and analyses from different countries. The major compounds are resins composed of flavonoids and phenolic acids or their esters, which often form up to 50% of all ingredients. The variation in beeswax content also influences the chemical analysis. In addition, it must be said that most studies do not attempt to determine all components but limit themselves to a class of chemicals or a method of extraction. The selection of the studies presented here is based on the most recent publications with preference given to the most complete studies or to studies from countries where these are the only references. Propolis is a vegetable mastic made by honeybees from resins collected on the bark and buds of certain trees and balsamic plants. The following uses of propolis, or its extracts, have been found in literature, but without substantiating evidence or reference to scientific studies. These are the anti-asthmatic treatment in mouth sprays, the support of pulmonary system, the anti-rheumatic (Donadieu, 1979) inhibition of melanoma and carcinoma tumor cells, the tissue regeneration, the strengthening of capillaries, the anti-diabetic activity, the phyto inhibitor, inhibiting plant and seed germination in general and potato and leaf salad seed germination (Bianchi, 1991) in particular. The composition of propolis varies from hive to hive, from district to district, and from season to season. Normally, it is dark brown in color, but it can be found in green, red, black, and white hues, depending on the sources of resin found

in the hive area. Honeybees are opportunists, gathering what they need from available sources, and detailed analyses show that the chemical composition of propolis varies considerably from region to region, along with the vegetation. In northern temperate climates, for example, bees collect resins from trees, such as poplars and conifers (the biological role of resin in trees is to seal wounds and defend against bacteria, fungi, and insects). "Typical" northern temperate propolis has approximately 50 constituents, primarily resins and vegetable balsams (50%), waxes (30%), essential oils (10%), and pollen (5%). Propolis also contains persistent lipophilic acaricides, a natural pesticide that deters mite infestations. In neotropical regions, in addition to a large variety of trees, bees may also gather resin from flowers in the genera *Clusia* and *Dalechampia*, which are the only known plant genera that produce floral resins to attract pollinators. *Clusia* resin contains polyprenylated benzophenones. In some areas of Chile, propolis contains viscidone, a terpene from *Baccharis* shrubs, and in Brazil, naphthoquinone epoxide has recently been isolated from red propolis, and prenylated acids such as 4-hydroxy-3, 5-diprenyl cinnamic acid have been documented. An analysis of propolis from Henan, China found sinapinic acid, isoferulic acid, caffeic acid, and chrysin, with the first three compounds demonstrating antibacterial properties. Also, Brazilian red propolis, largely derived from *Dalbergia ecastaphyllum* plant resin, has high relative percentages of the isoflavonoids 3-hydroxy-8, 9-dimethoxypterocarpan and medicarpin.

Other flavonoids commonly present include galangin and pinocembrin. Caffeic acid phenethyl ester (CAPE) is also a component of some varieties of propolis from New Zealand. Occasionally, worker bees will even gather various caulking compounds of human manufacture when the usual sources are more difficult to obtain. The properties of the propolis depend on the exact sources used by each individual hive; therefore, any potential medicinal properties that may be present in one hive's propolis may be absent from another's, or from another sample in the same hive. General medicinal uses of propolis include treatment of the cardiovascular and blood systems (anemia), respiratory apparatus particularly burn wounds, mycosis, mucous membrane infections and lesions), cancer treatment, immune system support and improvement, digestive tracts (ulcers and infections), liver protection and support and many others. (for various infections), dental care, dermatology (tissue regeneration, ulcers, eczema, wound healing. Some references to these applications can be found in the list of scientifically proven effects of propolis; otherwise one might refer again to IBRA's collection of abstracts, Apimondia and the American Apitherapy Society.

The composition of propolis depends on the type of plants accessible to the bees. Until 2000, over 300 chemical components belonging to the flavonoids, terpenes, and phenolics have been identified in propolis. The characteristic constituents in temperate region propolis are flavonoids without B-ring substituents, such as chrysin, galangin,

pinocembrin, pinobanksin. Caffeic acid phenethyl ester (CAPE) is a major constituent of temperate propolis with broad biological activities, including inhibition of nuclear factor κ-B; inhibition of cell proliferation; induction of cell cycle arrest and apoptosis. In tropical region propolis, especially Brazilian green propolis, the dominating chemical components are prenylated phenylpropanoids (e.g., artepillin C) and diterpenes. For propolis produced in the Pacific region, geranyl flavanones are the characteristic compounds which are also found in propolis from the African region (Fernandes-Silva*et al.*, 2013). The chemical composition of propolis is susceptible to the geographical location, botanical origin (Salatino *et al*., 2011; Toreti *et al*., 2013; Bankova, 2005; Silici and Kutluca, 2005), and bee species. To provide a theoretical basis to study the chemical composition and pharmacological activity of propolis and plant sources, and to control the quality, some chemical components were isolated for the first time from propolis between 2000 and 2012. They were scouted and summarized from databases, including BioMed Central, Biosis Citation Index, Medline, and PubMed. With the development of separation and purification techniques (such as high performance liquid chromatography (HPLC), thin layer chromatography (Alencar *et al.*, 2007), gas chromatography (GC), as well as identification techniques, such as mass spectroscopy (MS) (Campo*et al.*, 2008), nuclear magnetic resonance (NMR), gas chromatography and mass spectroscopy (GC-MS) Maciejewicz, 2001), more chemical compounds

ave been identified in propolis for the first time, including flavonoids, terpenes, phenolics and their esters, sugars, hydrocarbons and mineral elements. In contrast, relatively common phytochemicals such as alkaloids and iridoids have not been reported. Two hundred and forty-one (241) compounds have been reported for the first time from propolis between 2000 and 2012. Their chemical category, geographical locations, and possible plant source are summarized below. 3. Flavonoids As the major constituents of propolis, flavonoids contribute greatly to the pharmacological activities of propolis. The quantity of flavonoids is used as a criterion to evaluate the quality of temperate propolis (Zhang*et al.,* 2014). Flavonoids have a broad spectrum of biological properties, such as antibacterial, antiviral, and anti-inflammatory effects (Bueno-Silva*et al.*, 2013; Nijveldt*et al.*, 2001). According to the chemical structure, flavonoids in propolis are classified into flavones, flavonols, flavanones, flavanonols, chalcones, dihydrochalcones, isoflavones, isodihydroflavones, flavans, isoflavones and flavonoids. From 2000 to 2012, 112 flavonoids were identified in different type of propolis for the first time. In addition, flavonoid glycosides that are very rare in propolis were identified; they are isorhamnetin-3-O-rutinoside (Popova*et al.*, 2009) and flavone C-glycoside (Righi*et al.*, 2011). Five flavones 1–5 was identified in Chinese, Polish, Egyptian and Mexican propolis. According to the geographical origin and the typical chemical compounds, the botanical origins of these propolis samples are assumed to be the genus

Populus. In samples from the Solomon Islands and Kenya, researchers identified four flavonols 6–9 and confirmed that these compounds exhibited potent antibacterial activity (Inui*et al.*, 2013). Most of the identified compounds were also found in the plants. The characteristic constituents in temperate region propolis are flavonoids without B-ring substituents, such as chrysin, galangin, pinocembrin, pinobanksin. Caffeic acid phenethyl ester (CAPE) is a major constituent of temperate propolis with broad biological activities, including inhibition of nuclear factor κ-B; inhibition of cell proliferation; induction of cell cycle arrest and apoptosis. In tropical region propolis, especially Brazilian green propolis, the dominating chemical components are prenylated phenylpropanoids (e.g., artepillin C) and diterpenes. For propolis produced in the Pacific region, geranyl flavanones are the characteristic compounds which are also found in propolis from the African region (Fernandes-Silva*et al*., 2013). The chemical composition of propolis is susceptible to the geographical location, botanical origin (Salatino *et al*., 2011; Toreti *et al*., 2013; Bankova, 2005; Silici *et al*., 2005), and bee species (Silici *et al*., 2005). To provide a theoretical basis for studying the chemical composition and pharmacological activity of propolis and plant sources, and controlling the quality, chemical components that were isolated for the first time from propolis between 2000 and 2012 were scouted and summarized from databases including PubMed, BioMed Central, Biosis Citation Index and Medline, Chemical Compounds in Propolis. With the development of separation

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Flavonoids

As the major constituents of propolis, flavonoids contribute greatly to the pharmacological activities of propolis. The quantity of flavonoids is used as a criterion to evaluate the quality of temperate propolis (Zhang*et al.*, 2014). Flavonoids have a broad spectrum of biological properties, such as antibacterial, antiviral, and anti-inflammatory effects (Bueno-Silva*et al*., 2013; Nijveldt*et al.*, 2001). According to the chemical structure, flavonoids in propolis are classified into flavones, flavonols, flavanones, flavanonols, chalcones, dihydrochalcones, isoflavones, isodihydroflavones, flavans,

isoflavans and neoflavonoids. From 2000 to 2012, 112 flavonoids were identified in different type of propolis for the first time. In addition, flavonoid glycosides that are very rare in propolis were identified; they are isorhamnetin-3-*O*-rutinoside (Popova*et al.*, 2009) and flavone *C*-glycoside (Righi*et al.*, 2011). Five flavones - luteolin, 6-Cinnamylchrysin, 3', 5-Dihydroxy-4',7-dimenthoxy flavone, hexamethoxy flavone and (7''R)-8-[1-(4'-Hydroxy-3'-methoxyphenyl) prop-2-en-1-yl] chrysin - were identified in Chinese, Polish, Egyptian and Mexican propolis. According to the geographical origin and the typical chemical compounds, the botanical origins of these propolis samples are assumed to be the genus *Populus.* In samples from the Solomon Islands and Kenya, researchers identified four flavonols - 2'-(8''-Hydroxy-3'',8''-dimethyl-oct-2''-enyl)-quercetin, 8-(8''-Hydroxy-3'',8''-dimethyl-oct-2''-enyl)-quercetin, 2'-Geranylquercetin and macarangin -and confirmed that these compounds exhibited potent antibacterial activity (Inui*et al.*, 2012). Most of the identified compounds were also found in the plants *Macaranga*, suggesting that the genus *Macaranga,* is the likely plant source. In Pacific propolis, scientists identified many prenylated flavanones (5,7,3',4'-Tetrahydroxy-5'-C-geranylflavanone,5,7,3',4'-Tetrahydroxy-6-C-geranylflavanone, 5,7,3',4'-Tetrahydroxy-2'-C-geranylflavanone, 5,7,3',4'-Tetrahydroxy-2'-C-geranyl-6 prenlyflavanone, Propolin A, Propolin B, Propolin E, Sigmoidin B, Bonannione A, Solophenol A, Sophoraflavanone) which exhibited strong antimicrobial activity because the

lipophilic prenyl group could rapidly damage the membrane and cell wall function (Aghukumar*et al*., 2010). Some flavanones - 3-O-[(S)-2-Methylbutyroyl] pinobanksin, Hesperitin-5,7-dimethyl ether, Pinobanksin-5-methyl-ether-3-O-pentanoate, (2R,3R)-3,5-Dihydroxy-7-methoxyflavanone 3-(2-methyl)-butyrate -were also identified in poplar propolis. Sherstha *et al.* identified threeflavanonols- (2R, 3R)-3,6,7-Trihydroxyflavanone, 5-Methoxy-3-hidroxyflavanone, 5,7-Dihydroxy-6-methoxy-2,3-Dihydroflavonol-3-acetatein Nepalesepropolis, Portuguese propolis and Australian propolis, respectively.

Terpenoids

Although volatiles only represent 10% of the propolis constituents, they account for the characteristic resinous odor and contribute to the pharmacological effects of propolis. As the major compounds among the volatile substances, terpenoids play an important role in distinguishing premium propolis from inferior or fake propolis and they exhibit antioxidant, antimicrobial, and other biological activities. Monoterpenes isolated from propolis include acyclic, monocyclic, dicyclic monoterpenes and their derivatives. The primary acyclic and monocyclic monoterpenes are myrcenes, *p*-menthanes and cineoles, respectively. The cyclic monoterpenes in propolis are classified into five groups: thujanes, caranes, pinanes, fenchanes and camphenes. Sesquiterpenes Are the most abundant chemical components in propolis. According to the number of the rings,

sesquiterpenes fall into four categories: acyclic, monocyclic, bicyclic and tricyclic. The main acyclic sesquiterpenes in propolis are the derivatives of farnesane. There are four types of monocyclic sesquiterpenes, five types of dicyclic sesquiterpenes and ten types of tricyclic sesquiterpenes in propolis. Cembrane, labdane, abietane, pimarane, and totarane are reported to be the major diterpenes in propolis, and some of these are proven to have a broad spectrum of pharmacological properties. The tetracyclic triterpenes in propolis are lanostanes and cycloartane and the pentacyclic triterpenes are oleanane, ursane andlupane. One monoterpene (*trans*-β-terpineol) and three sesquiterpenes (γ-elemene, α-ylangene, valencene) with valuable biological activities were identified in Brazilian propolis (Oliveira*et al.*, 2010). In Turkish propolis, a few sesquiterpenes- 8-βH-Cedran-8-ol, 4-βH,5α-Eremophila-1(10)-ene, α-Bisabolol, α-Eudesmol, α-Cadinol- were identified; and there was no direct evidence to determine the correct plant source of each type of Turkish propolis (Kartal*et al.*, 2002). Popova *et al.*(2009) identified the usual "Mediterranean" diterpenes in samples from Greece, together with some diterpenes that are deemed as characteristic oleoresin components of different *Coniferae* (mainly *Pinaceae* and *Cupressaceae*) plants, although their plant source was considered to be the *Cupressaceae* because Greek propolis contained ferruginol, totarol, oxygenated ferruginol and totarol derivatives, and semperviren, which are typically found in Cupressaceae plant, but not in Pinaceae. Some triterpenes belonging to the lupane (lupeol

alkanoates, lupeol, lupeol acetate),lanostane(lanosterol acetate, lanosterol),oleanane(germanicol acetate, germanicol, β-amyrin acetate),ursane(β-amyrone, α-amyrin acetate, α-amyrone) andothertypes (24-Methylene-9, 19-ciclolanostan-3β-ol, (22Z,24E)-3-Oxocyclohex-22, 24-dien-26-oic acid, (24E)-3-Oxo-27, 28-dihydroxycyclo-art-24-en-26-oic acid, 3,4-seco-Cycloart-12-hydroxy-4 (28), 24-dien-3-oic acid, Cyclo-art-3,7-dihydroxy-24-en-28-oic acid, 3-Oxo-triterpenic acid methyl ester) were found in Brazilian, Cuban, Greek, Burmese and Egyptian propolis for the first time.

Phenolics

Brazilian green propolis is rich in phenylpropanoids including cinnamic acid, p-coumaric acid, caffeic acid, ferulic acid and their derivatives. Among these substances, prenylated cinnamic acids turn out to be a salient chemical feature and have a consanguineous bearing on antimicrobial activity of green propolis. In recent years, researcher sidentifiedaserieso fphenyl propanoid derivatives (cis-3-Methoxy-4-hydroxycinnamic acid, trans-3-Methoxy-4-hydroxycinnamic acid, 3-Prenyl cinnamic acid allyl ester, p-Methoxycinnamic acid, Dihydroxycinnamic acid, 3-Prenyl-4-hydroxycinnamic acid, 3,5-Diprenyl-4-hydroxycinnamic acid, 3-Methyl-2-butenyl isoferulic, 3-Methyl-3-butenyl caffeate, Hexadecyl caffeate)in Brazilian propolis. Meanwhile, some caffeic acid derivatives - tetradecenyl caffeate (isomer), tetradecenyl caffeateand isoferulic acid derivative (2-Methyl-2-butenyl ferulate) were also identified in poplar

propolis by GC-MS. Chlorogenic acid is abundant in Brazilian Propolis Of floral origin from *Citrus* spp. (Dos Santos Pereira*et al.*, 2003). Three quinic acid derivatives - 4-Feruoyl quinic acid, 5-Feruloyl quinic acid and 3,4,5-tri-O-Caffeoylquinic acid were identified in this type of propolis. Another class of phenolics, stilbenes, are not very common in plants. In 2010, Petrova*et al* .(2010)identified two geranyl stilbenes; schweinfurthin A and schweinfurthin B in propolis produced in Kenya.

In 2012, another stilbene, 5 - farnesyl - 3' - hydroxyresveratrol was identified in Solomon Island propolis, which is also present in Macaranga plants (Inui*et al*., 2010). These results suggest that *Macaranga* is probably the plant source of the propolis from Kenya and Solomon Island. However, many stilbenes (5, 4' - Dihydroxy - 3' - methoxy - 3 - prenyloxy - E - stilbene, 3, 5, 3', 4' - Tetrahydroxy - 2 - prenyl - E - stilbene, 3, 5, 4' - Trihydroxy - 3' - methoxy - 2 - prenyl - E - stilbene, 5, 3', 4' - Trihydroxy - 3 - methoxy - 2 - prenyl - E - stilbene, 5, 4' - Dihydroxy - 3, 3' - dimethoxy - 2 - prenyl - E - stilbene, 5, 4' - Dihydroxy - 3 - prenyloxy - E - stilbene, 3', 4' - Dihydroxy - E - stilbene, 3', 4' - Dihydroxy - 3, 5 - dimethoxy - E - stilbene, Diprenylated dihydrostilbene, 3, 5 - Dihydroxy - 2 - prenyl - E - stilbene, 4 - Prenyldihydroresveratrol, 3 - Prenylresveratrol), especially prenylatedstilbenes, were identified in Australian Kangaroo Island propolis, which makes this type of propolis a stronger scavenging activity towards DPPH free radical than Brazilian propolis (Abu - Mellal*et al.*, 2012), suggesting the source of stilbenes is not limited to only a fewplants.

Lignansas main chemical compounds in tropical propolis have attracted a worldwide research interest. In the past 12 years, researchers identified three lignans (Tetrahydrojusticidin B, 6 - Methoxydiphyllin, Phyllam ricin C) in Kenyan and Brazilian propolis. As shown in table below, other phenolic compounds and derivatives were identified in propolis from Brazil(8 - (Methyl - butanechromane) - 6 - propenoic acid, 3 - Hydroxy - 2, 2 - dimethyl - 8 - prenylchromane - 6 - propenoic acid, 2, 2 - Dimethyl - 8 - prenylchromene - 6 - propenoic acid, 2, 2 - Dimethylchromene - 6 - propenoic acid, 2, 2 - Dimethyl - 6 - carboxyethnyl - 2H - 1 - benzopyran, 2, 2 - Dimethyl - 6 - carboxyethenyl - 8 - prenyl - 2H - 1 - benzopyran, Nemorosone, 7 - epi - clusianone, Xanthochymol, Gambogenone, Hyperibone A), Indonesia(5 - Pentadecylresorcinol, 5 - (8'Z, 11'Z - Heptadecadienyl) - resorcinol, 5 - 11'Z - Heptadecenyl) - resorcinol, 5 - Heptadecylresorcinol, 1, 3 - Bis (trimethylsilylloxy) - 5, 5 - proylbenzene, 3, 4 - Dimethylthioquinoline, 4 - Oxo - 2 - thioxo - 3 - thiazolidinepropionic acid, D - glucofuranuronic acid, Dofuranuronic acid, 3 - Quinolinecarboxamine), France(Baccharin), Iran(Suberosin, Tschimgin, Tschimganin, Bornyl p - hydroxybenzoate, Bornyl vanillate, Ferutinin, Tefernin, Ferutinol p - hydroxybenzoate, Ferutinol vanillate) andMalta(2 - Acetoxy - 6 - p - methoxybenzoyl jaeschkeanadiol, 2 - Acetoxy-6-p-hydroxybenzoyl jaeschkeanadiol).Among these chemicals, nemorosone (Nemorosone) is the exclusive and principal component of *Clusia rosea* floral resins, indicating that *Clusia* spp. is the plant origin of the brown propolis (Camargo*et*

al., 2013). Tschimgin, tschimganin, ferutinin, tefernin identified in Iranian propolis are the characteristic compositions of the *Ferula* species, which is considered as another plant source of Iranian propolis besides poplar.

Sugars

The question about the origin of sugars in propolis has not been solved yet. Nectar and honey are thought to be the sources of glucose, fructose, and sucrose. Others suggest that they come from hydrolyzed flavonoid glycosides in propolis. In addition, mucilages containing numerous sugars, sugar alcohols and acids were listed among potential propolis sugar sources by Crane (1988). In the propolis originated from the Canary Islands and Malta, many sugars, sugar alcohols and uronic acids were identified, supporting the claim that plant mucilages were the source of these compounds (Popova*et al.*, 2011). In Egyptian propolis, many sugars, sugar alcohols and uronic acids were identified by GC-MS. Among These substances, galactitol, gluconic acid, galacturonic acid and 2-O-glyceryl galactose were identified in propolis for the first time (El Hady and Hegazi, 2000).

Hydrocarbons

Hydrocarbons are other basic components of propolis. In recent years, alkanes, alkenes, alkadienes, monoesters, diesters, aromatic esters, fatty acids and steroids have been identified in many types of propolissuchasEgyptianpropolis(Hegazi

and El Hady, 2002),Brazilian Propolis(Teixeira*et al.*, 2005) andAnatolianpropolis(Uzel*et al.*, 2005).Comparing the compositions of Brazilian propolis waxes and comb waxes which were produced by the same colony, no difference was found to allow a distinction, suggesting a common origin for both wax sources (Negri*et al.*, 2000). This result not only illustrates that propolis waxes are secreted by bees (Negri 1998), but also indicates that the composition of propolis waxes and comb waxes is only dependent on genetic factors of the bees, not plant sources.

Minerals

Trace Elements (Ca, K, Mg, Na, Al, B, Ba, Cr, Fe, Mn, Ni, Srand Zn) and toxic elements (As, Cd, Hg and Pb) were discovered by atomic emission/absorption spectrometry in propolis samples collected from different Croatian regions (Cvek*et al.*, 2008). Br, Co, Cr, Fe, Rb, Sb, Sm and Zn were identified in different Argentinean propolis by neutron activation analysis. These studies show that the trace element profiles can be useful for propolis identification according to their location (Cantarelli*et al.*, 2011).

The Chemical Categories Reported in Propolis

The chemical categories reported in propolis during 2000 and 2012 are summarized in below table and graph, indicating consistency with the categories reported previously. It is well recognized that the chemical composition of herbal medicines is

affected by many environmental factors while maintaining their genetic characteristics (Razmovski-Naumovski*et al.*, 2010). Similar effects to propolis can be expected from environmental factors. However, bee species need to be considered together with geographical factors and plant sources.

Bee Species and Propolis

We propose that species, subspecies, and varieties of bees have a major impact on the chemical components and quality of propolis. The genus *Apis* contains 10 generally recognized species. Honeybee, *A. mellifera*, is widely spread in Europe, Ural Mountains, Africa, and Asia. All other recognised *Apis* species are of Asian distribution. About 25 subspecies have been recognized for*A. mellifera*, based on morphometry, behaviour, and biogeography (Arias and Sheppard, 2005) belonging to three or four major subspecies groups (Arias and Sheppard, 1996). The most popular species of honeybee is the European honeybee, *Apies mellifera*. It has been shown that varieties of bee affect the antibacterial activity of propolis collected from thes ameapiary; *A. melliferacarnica*hives showed weaker antibacterial activity than that of *A. mellifera anatolica* and *A. melliferacaucasica.* The three honeybee races used neither the same nor the single plant source (Silici and Kutluca, 2005). In another type of propolis, geopropolis, produced by stingless bee species, *Melipona scutellaris*, benzophenones, but no flavonoids, have been identified as the major compounds (Da Cunha*et al.*,

2013); However,geo propolis producedby *Meliponafasciculate* contains high concentrations of polyphenols,flavonoids, triterpenoids, saponins, and even tannins(Dutra*et al.*, 2014).Although different species of honeybee preferdifferentplants,thechemicalprofileofpropolisthatis produced by the same species is not always same. Brazilian green and red propolis both originate from Africanized *A. mellifera* (Teixeira*et al.*, 2005; Daugsch*et al.*, 2008), but these propolis are rich in prenylated phenylpropanoids and isoflavonoids respectively. The differences are due to the plants, namely *B. dracunculifolia* and *Dalbergia ecastophyllum,* which are used by bees as resin sources. In cerumen propolis from stingless bees (*Tetragonula carbonaria*), *C*-methylated flavanones, terpenic acids and phenolic acids, such as gallic acid, diterpenic acids of pimaric and abietic type are the predominant chemicals, but it lacks the characteristic flavonoids and prenylated phenolics found in propolis from honeybees' species in Australia(Massaro*et al.*, 2011; Massaro*et al.*, 2014].Therefore, the variant chemical composition of propolis depends on the bees' preferences of botanical sources and the species and varieties of bees(Leonhardt *et al.*, 2010; Leonhardt and Blüthgen, 2009; Leonhardt*et al.*, 2009).

The Geographical Origins of Propolis

Propolis collected from many countries have demonstrated chemical profiles like the poplar type propolis: China (Ahn *et al.*, 2007), Korea, Croatia (Kosalec *et al.,* 2003),

different regions of Taiwan (Chen *et al.*, 2003; Chen, 2004; Huang *et al.*, 2007), New Zealand (Markham *et al.*, 1996) and Africa (Hegazi and El Hady, 2002). Poplar tree (*Populus nigra* L. and *P. alba* L) is common in Europe and is used to name the common type of propolis that is rich in flavonoids and phenylpropanoids. However, flavonoids are not restricted to poplar; furthermore, in areas where poplars are not native plants, such as Australia and equatorial regions of South America, bees will seek other plants to produce propolis, which contain the flavonoids of the poplar type propolis (Li *et al.*, 2010). Propolis from the tropical zone, Brazilian green and red propolis, are respectively rich in prenylated derivatives of p-coumaric acid, and some isoflavonoids that are different from the ones found in poplar type propolis (Bankova *et al*., 2000; Trusheva *et al.*, 2007). In addition, propolis from Solomon Island, Burma, Greek, and Japan are characterized by the geranylated and prenylated flavonoids.

The Plant Sources of Propolis

The current opinion is that propolisis collected from resin soft rees such as poplars and conifers, and therefore propolis is sometimes classified after the name of the source plant (Kosalec *et al*., 2004; Bankova *et al*., 2000; Burdock, 1998). The plant source is identified by observing the collection activities of bees and comparing the chemical profiles of propolis and plant materials. Other researchers found that honeybees collect plant material by cutting fragments of vegetative tissues, so the

anatomical characteristics of plant tissue in the propolis can be used as evidence of propolis origin (Teixeira*et al.*, 2005). As in the last section, *Populus* species are the main plant origin of propolis all over the world, especially in the temperate zone. Most propolis collected from Europe, North America, non-tropical region of Asia, New Zealand (Bankova *et al*., 2000) and even Africa (mainly the east area of Nile Deltaregion) (Hegazi and El Hady, 2002) contains the characteristic poplar chemical profile: high level of flavanones, flavones, low phenolic and their esters (Mohammadzadeh*et al*., 2007). In the tropical and subtropical area, there are few poplar trees. Honeybees must search for a new plant source for propolis. For the propolis collected from south east of Brazil, *Baccharisdracunculifolia* turns out to be the main botanical source [66, Kumazawa *et al*., 2003). Artepillin Casthesalient chemical composition makes it easy to distinguish this propolis from other types of propolis. It is reported that propolis from Venezuela, Amazon and Cuba contains prenylated benzophenones, which originated from the exudates of *Clusia* flower (De Castro Ishida*et al.*, 2011; Trusheva*et al*., 2004). *Macaranga* plants have been demonstrated to be the plant source of Taiwan (Huang*et al.*, 2007), Okinawan that was classified as Pacific propolis (Bankova *et al*., 2000). High concentration of diterpenoids in Mediterranean propolis may originate from *Cupressus*plants for Sicilian, Cretanpropolis (Popova*et al.*, 2009) and Malteseпropolis (Popova*et al.*, 2011), *Pinus* plants for Greek propolis (Melliou and Chinou, 2004). In Kangaroo Island

(Australia), bees collect propolis from the sticky exudate on the stem shoots and seed pods of an endemic Australianplant, *Acaciaparadoxa* (Tran*et al.*, 2012). Red Brazilian propolis and Nepalese propolis have various biologically active flavonoids that primarily come from the genus *Dalbergia* (Alencar*et al*., 2007; Awale*et al*., 2005). However, some of plant sources are just surmised by observing the bees' foraging behaviors, not comparing chemical identity of secondary plant metabolites in propolis and in the plant source. For example, *Eucalyptus* species are considered as the source plant in Australia, southAnatolia (Turkey) (Silici*et al.*, 2007), Ismailia (Egypt) (El Hady and Hegazi, 2000) and Brazil, but no real proof has been presented for this origin. Therefore, its till needs further study to compare chemical compounds in propolis and the plants, to confirm the exact botanic origin.

Summary and Future Perspectives

The biological activities of propolis are attributed to a variety of major chemical constituents including phenolic acids, phenolic acid esters, flavonoids, and terpenoids, such as CAPE, artepillin C, caffeic acid, chrysin, and galangin quercetin, apigenin, kaempferol, pinobanksin 5-methyl ether, pinobanksin, pinocembrin, pinobanksin 3-acetate. *Over 500 compounds have been identified in propolis from many countries up to 2012.* They belong to flavonoids, phenylpropanoids, terpenoids, stilbenes, lignans, coumarins and their prenylated derivatives. However, other common

chemical components such as alkaloids, iridoids have not been reported in propolis. This characteristic is often explained by the plant sources. We recommend that bee varieties and subspecies need to be considered together with geographical factors and plant species around the beehive in future studies on propolis. The priorities of future research lie on the influence of species and behavior on propolis, together with feeding experiments to identify the plant part source, which will advance our understanding of the chemistry and quality of propolis, as well as honey bee biology. Characterization of propolis from various locations and plant sources is warranted to define acceptable quantitative standards for different types of propolis. Furthermore, the biological activities of each type of propolis need to be correlated with their chemical composition, and eventually, standardized products should be used in clinical studies. Propolis changes in color, odor, and probably medicinal characteristics, according to source and the season of the year. Moreover, some bees and some colonies are more avid collectors-generally to the dismay of the beekeeper, since propolis is a very sticky substance which, in abundance, can make it difficult to remove frames from the boxes. Foraging for propolis is only known with the Western honeybee *Apis mellifera*. The Asian species of Apis do not collect propolis. Only Meliponine or stingless bees are known to collect similarly sticky resinous substances, for sealing hives and constructing honey and pollen pots for storage. In this bulletin, however, propoli shall refer only to

resins collected by honeybees, since almost all the research has been done on it. There may well be similar traditional uses for resins collected by Meliponids. In the natural distribution ranges of *Apis mellifera*, a multitude of traditional uses are known for this versatile substance.

The Greeks and Romans already knew that propolis would heal skin abscesses and through the centuries its use in medicine has received varying attention. The ancient Egyptians knew about the benefits of propolis and in Africa it is still used today, as a medicine, an adhesive for tuning drums, sealing cracked water containers or canoes and dozens of other uses. It has been incorporated in special varnishes such as those used by Stradivarius for his violins (Jolly, 1978).

Collection, Processing and Storage of Propolis

Contamination of propolis on candles, paints and other parts should be avoided. The cleanest collection method uses traps placed on top of the hive. Traps are plates with small holes that essentially resemble chambers or cracks in the hive wall. Bees try to close these holes to protect their hives from external factors and thus fill the hive with propolis. Thanks to the traps, excess wax does not mix with the propolis, and contamination does not occur during harvest. Harvesting with traps is a faster and more efficient method. To increase propolis production, traps are made of plastic, nylon, or metal with openings wide enough (3 mm) that the bees cannot pass through until the weather cools down. Traps are mounted on

the top of the hive. The openings on the traps are filled with propolis by the working bees for 12 to 21 days.

Photo 9. Collecting propolis

Quality Control in Propolis

Propolis is effective in cleaning the honeycomb cells, developing the eggs laid by the queen in a sterile environment, and protecting the offspring. Propolis is also used by bees to shape the edges of the hive, harden, and repair the edges of the honeycomb, strengthen the frame connections, fix the frames in the hives, close the cracks and cracks, and collect them for these purposes. The reason why microorganisms are much less abundant in the hive than in the atmosphere is the presence of propolis in the hive. When the inner walls of the hive are plastered with propolis, they become slippery, and it becomes

easier for the bees to repel ants that try to enter the hive. Various insects that enter the hive and die or other particles that cannot be removed from the hive are covered with propolis to prevent them from damaging the hive. It keeps the humidity in the hive at a certain level and protects the hive from excessive humidity that occurs after heavy rains. It ensures the hygiene of the hive by preventing the development of various spores and the like. However, lack of standardization limits the use of propolis. For this reason, countries have started to create their own standards.

Processing of Propolis

Cool autumn weather comes at a time when the leaves are changing color, mice are nesting in well-protected warm places, and bees are plugging the cracks in their hives with propolis in anticipation of winter. The term propolis (aka bee glue) originated with the Greeks, who often observed a sticky resinous substance at the entrance to their hives. In Greek, “Pro” means to come before or in front of, and “Polis” is Greek for city or a body of citizens. Therefore, propolis is what one might expect to find at the entrance to the city of bees. Today, beekeepers will often observe bees using propolis to restrict or narrow the hive entrance to facilitate defense. Honey Bees use propolis both as a building material and to sterilize and disinfect the space where the colony is located. Because, as we will examine in this two-part series, propolis is one of the most powerful antimicrobial substances

found in nature. Honey Bees make propolis from resins they collect from deciduous trees such as birch, alder, and poplar. As these trees bud, they release these resins around the bud to protect against fungi and other diseases. Foraging bees use pollen baskets (corbiculae) to transport propolis resin spheres back to the hive. However, unlike pollen, foragers require the help of other bees within the colony to help them remove the sticky resin from their hind legs so that it can be used by the colony. While propolis is found in many products, from toothpaste and skin creams to healing ointments, herbal tinctures, syrups, and potions, propolis does not require any processing (other than cleaning) to be used. For gum, tooth, or sore throat problems, insert some raw propolis between the gum and cheek and suck it. This is the simplest way to use it, although its benefits are limited, and it can stick to your teeth if you are not careful.

Photo 10. Storage of propolis in glass jars in small-scale family businesses

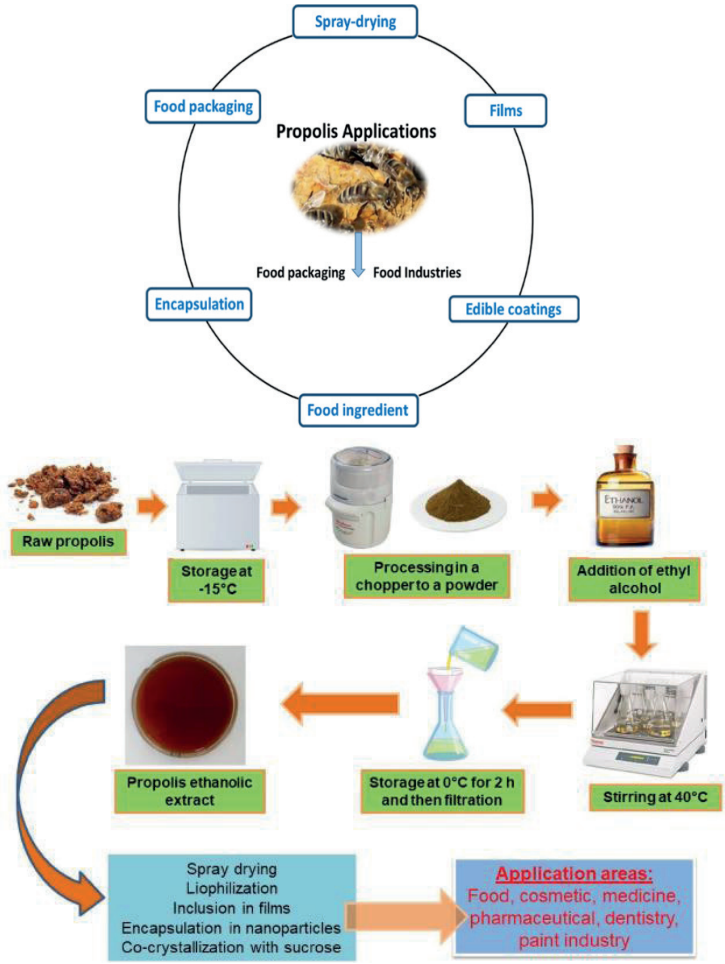

Fig. 20. Propolis applications in food industries and packaging

Check Yourself

Propolis is mainly a mixture of various amounts of:

a) vitamins and minerals
b) beeswax and resins
c) proteins and lipids
d) essential amino acids and non-protein nitrogen compounds

1. The chemical composition of propolis is susceptible to:

a) the geographical location
b) botanical origin
c) bee species
d) all answers are correct.

2. Flavonoids:

a) exhibit antibacterial activity.
b) exhibit antifungal activity.
c) exhibit anti-inflammatory activity.
d) all answers are correct.

3. Chalcones are:

a) terpenes
b) phenolics
c) flavonoids
d) no answer is correct.

4. How many different chemical structures are there in the propolis?

a) 100
b) 300
c) 200
d) 225

5. Propolis shows an elastic structure of wax consistency between 15-25ºC, softens and becomes sticky at high temperatures. What is the partial melting temperature of propolis?

a) 80
b) 60
c) 45
d) 55

6. Volatiles, with the major terpenoids contribuing to the pharmacological effects of propolis represent around?

a) 10% of the propolis constituents
b) 20% of the propolis constituents
c) 5% of the propolis constituents
d) 50% of the propolis constituents

7. The most abundant chemical components in propolis are:

a) monoterpenes
b) diterpenes
c) sesquiterpenes
d) flavons

8. It has been shown that antibacterial activity of propolis:

a) of *A. mellifera carnica* is **lower** than of *A. mellifera anatolica*
b) of *A. mellifera carnica* is **higher** than of *A. mellifera caucasicas*
c) of *A. mellifera anatolica* of is **lower** than of *A. mellifera carnica*
d) the antibacterial activity of propolis does not depends on honeybee species

9. Geopropolis, produced by stingless bee species, *Melipona scutellaris* contains:

a) no benzophenones
b) no flavonoids
c) no terpens
d) no phenolice)

Answers: 1b, 2d, 3d, 4c, 5b, 6b, 7a, 8a, 9a, 10b

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Bee Venom

Dr. Barbara KRÓL *Dr. Maja SŁUPCZYŃSKA*
Wrocław University of Life and Environmental Sciences, Wrocław, Poland

Bee Venom and its Composition

Among the many species of insects, only very few have the capability of defending themselves with a sting and venom injection during stinging. All insects that can sting are members of the order Hymenoptera, which includes ants, wasps, and bees. Since the sting is believed to have evolved from the egg-laying apparatus of the ancestral, hymenopteran species, only females can sting. The sting is always at or near the abdominal end, rather than the head. Therefore, the pain inflicted by a honeybee, defending its colony, is not caused by a bite, as is frequently said, but by a sting. There are many other poisonous insects which secrete venom. They usually cover their body with it, spray it, create wounds, and secrete it into the wound, or inject it via mouthparts or a sting. In some cases, the venom is used for defense of the individual or, in the case of social insects, the colony. But venom is also used in killing prey (as with some wasps or spiders) or for immobilizing and preserving prey (for their own or their developing offspring's consumption). Honeybee venom is

produced by two glands associated with the sting apparatus of worker bees. Its production increases during the first two weeks of the adult worker's life and reaches a maximum when the worker bee becomes involved in hive defence and foraging. It diminishes as the bee gets older. The queen bee's production of venom is highest on emergence, probably because it must be prepared for immediate battles with other queens.

Bee venom (apitoxin)is a clear, odorless, watery liquid a bitter taste and basic pH (4.5 to 5.5). When meeting mucous membranes or eyes, it causes considerable burning and irritation. Dried venom takes on a light-yellow colour. Bee venom is synthesized in the venom glands of worker bees and queen. Venom is pro-duced by two glands associated with the sting apparatus of worker bees and is stored in the venom sac. In workers bee venom is a unique weapon and has a prime role in bee colony defense and foraging. The queen bee's production of venom increases in emergence, probably because it must be prepared for battles with other queens. A mature defender or forager con-tains about 100-150 µg of venom, and it inject 0.15 – 0.30 mg of venom via its stinger, a honeybee can inject 0.1 mg of venom via its stinger and the young queen contains about 700 µg. There are more than 60 identifiable components in bee venom, and melittin is the most prevalent substance. The honeybee venom consists of enzymes, proteins, peptides and a va-riety of smaller molecules: amino acids, catecholamines, sugars, and minerals. Most types of venom induce immediate pain because they contain phospholipases, hyaluronidase, and

other enzymes. Venom composition from various Apis species is quite similar butmay slightly vary from each other. It was stated that the toxicity of *Apis cerana* venom is twice as high as that of *Apis mellifera*. Characteristic of the most important bee venom substances and its classification is presented in tables below.

Class of molecules	Components
Enzymes	**Phospholipase A2** (PLA2) - enzyme hydrolysing phospholipids; **Phospholipase B** - enzyme doing cleavage of the toxic lysolectin; **Hyaluronidase** - catalyses hydrolysis of hyaluronic acids, the tissue cement; **Acid Phosphomonoesterase; Phosphatase** (acid and alkaline); **Lysophospholipase**; **α-Glucosidase**.
Peptides	**Melittin** - biologically most active peptide; **Apamine** - biologically active peptide; **Mast Cell Degranulating** Peptide (MCD) - mast cell degranulating peptide; **Secapine**, **Pamine**, **Minimine** - small peptides of less than 5 amino acids; **Adolapin** - biologically active peptide; **Protease inhibitor** - biologically active peptides; **Procamine A, B**; **Tertiapine, Cardipopep**.
Active amines	**Histamine**; **Dopamine** (DA); **Noradrenaline**; and neurotransmitters.
Amino Acids	**γ-amino-butyric acids** and **α- amino acids**.
Sugars	**Glucose** and **Fructose**.
Minerals	P, Ca, Mg
Volatile Compounds	**Complex ethers, iso-pentyl acetate; n-butyl acetate; iso-pentanol; n-hexyl acetate; noctyl acetate; 2-nonanol; n-decyl acetate; benzyl acetate; benzyl alcohol; (2)-11-eicosen-1-ol.**

Table 8. Honeybee venom composition (according to Banks and Shipolini, 1986; Dotimas and Hider, 1987; Shkenderov and Ivanov, 1983; Urtubey, 2005)

Class of molecules	Component	% of dry venom[a]	% of dry venom[b]
Enzymes	Phospholipase A_2	10-12	10-12
	Hyaluronidase	1-3	1.5-2.0
	Acid		1.0
	Phosphomonoesterase		1.0
	Lysophospholipase		0.6
	a -glucosidase		
Other proteins and peptides	Melittin	50	40-50
	Pamine	1-3	3
	Mast Cell	1-2	2
	Degranulating Peptide	0.5-2.0	0.5
	(MCD)	1-2	1.4
	Secapin		1.0
	Procamine	0.1	0.8
	Adolapin	13-15	0.1
	Protease inhibitor		
	Tertiapin		
	Small peptides (with less than 5 amino acids)		
Physiologically active amines	Histamine	0.5-2.0	0.5-1.6
	Dopamine	0.2-1.0	0.13-1.0
	Noradrenaline	0.1-0.5	0.1-0.7
Amino Acids	t -aminobutyric acid	0.5	0.4
	a -amino acids	1	
Sugars	Glucose & fructose	2	
Phospholipids		5	
Volatile compounds		4-8	

Table 9. Composition of venom from honeybee worker

Bee Venom Bioactive Substances

Melittin

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. Chemically, 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 is a surfactant; it causes hemolysis of erythrocytes, releases histamine from mast cells, and 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. It has strong surface effects on cell membranes causing pore-formation in epithelial cells and the destruction of red blood cells. Melittin also activates nociceptor (pain receptor) cells. However, in honey bees, melittin is not only expressed in the venom gland, but also in other tissues when bees are infected with various pathogens. It indicates that melittin may play important role in the immune response of bees to infectious diseases. The physicochemical properties of melittin cause its pronounced antibacterial activity against many species of microorganisms, including mycoplasma. The main biological function of melittin is listed in the table below.

Major function: hemolytic activity, anti-inflammatory activity, anticancer, antibacterial, antifungal, antiviral activities.
Other function: inhibits well-known transport pumps (such as Na+-K+-ATPase, H+-K+-ATPase), activates phospholipase A2, diminishes membrane surface tension, stimulates smooth muscle, lowers blood coagulation, influences central nervous system (CNS), Increases capillary permeability.
Adverse effects: initiates various allergic reactions, lyses erythrocytes, creates cytotoxicity in human peripheral blood lymphocytes, and modulates gene expression related to apoptosis, DNA damage response and oxidative stress.
Medical applications: arthritis, cancer, diseases of central and peripheral nervous systems, skin diseases, heart and blood system related diseases, frozen shoulder, asthma, bronchitis, colitis, ulcers, ophthalmology, endocrinology, urology, gynecology, otorhinolaryngology

Biological and therapeutic effect of melittin results from its effect on membrane-active; it diminishes surface tension of membranes and stabilizes them. Melittin has anti-inflammatory effect in very small doses; it stimulates smooth muscles, activates the hypophysis and adrenal glands, increases capillary permeability, increasing blood circulation and lowering the blood pressure, and lowers blood coagulation. It has immunostimulatory and immunosuppressive effect. Additionally, melittin influences the central nervous system. It has anticancer, antibacterial, antifungal, antiviral, anti atherosclerosis, endosomolytic (helps packaging components for gene therapy) activity as well. Higher doses of melittin are inflammatory and haemolytic, and the **toxicity measured in rat experiments is 4 mg/kg.**

Apamin

Apaminis, 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 is strongly basic like melittin but in contrast to melittin apamin shows no cytolytic activity against human erythrocytes. 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. The **LD_{50} 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 Ca^{2+} activated K^{+} channels (SK channels) in neurons. These channels are responsible for the afterhyperpolarizations that follow action potentials, and therefore regulate the repetitive firing frequency. SK channels do not only regulate afterhyperpolarization, but they also influence synaptic plasticity - an important mechanism underlying learning and memory processes. Apamin is expected to influence these processes by inhibiting SK channels. This may provide a basis for the use of apamin as a treatment for memory disorders and cognitive dysfunction. SK channel blockers, as apamin, may have a therapeutic effect on Parkinson's disease. Dopamine, which is depleted in this disease, will be released from midbrain dopaminergic neurons when these SK channels are inhibited. SK channels have also been proposed as targets for the treatment

of epilepsy, emotional disorders, and schizophrenia. Due to the risk of toxic effects, the therapeutic window for apamin is very narrow.Biological and therapeutic effect of apamin includes anti-inflammatory stimulating the release of cortisone, antiserotonine action, preserves red blood cells. It increases the defence capability, anticomplementary, activates the hypophysis and adrenal glands. Apamin is an immunosuppressant and has specific effects in the brain that might be linked to Alzheimer and multiple sclerosis (MS) diseases. As it was mentioned above in might has anti-Parkinson effect. Higher doses of apamin are neurotoxic; the toxicity measured in rat experiments is 4 mg/kg.

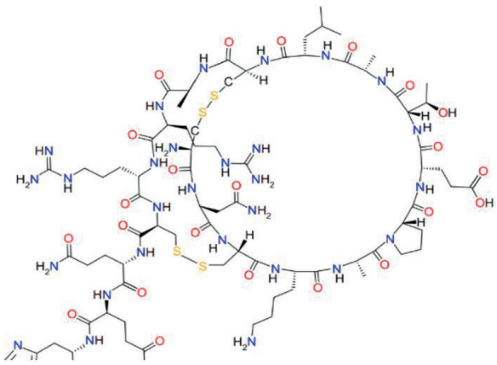

Fig. 21. Structure of apamin

Adolapin

Adolapinis polypeptide with 103 amino acid residues and comprising 1% of dry matter of bee venom molecular mass

proved to be 11500 - 11092 Da. Adolapin exhibited a potent analgesic effect demonstrated by the "writhing" test and by the Randall-Sellito's test. Adolapin shows anti-inflammatory and antinociceptive properties. Its action involves the inhibition of both prostaglandin synthase *via* inhibition of cyclooxygenase activity and lipoxygenase in human platelets. This peptide also has antipyretic effects and inhibits the increase in mean body temperature.

Biological and therapeutic effects of adolapin include inhibition of the specific brain enzymes: cyclooxygenase and lipoxygenase. It inhibits the aggregation of erythrocytes, decreases inflammation, has anti-rheumatic and antipyretic properties as well as decreases pain. Adolapin has relatively low toxicity - the toxicity measured in rat experiments is 40 mg/kg.

Phospholipase A2 (FLA2)

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. It can hydrolyze phospholipids, resulting in the formation of lysolecithin, which has a cytolytic effect. It can lyse membranes of many cells

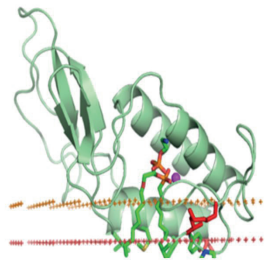

Fig. 22. Structure of phospholipase A2 (FLA2)

(erythrocytes, mast cells) thereby providing a manifestation of pathological effects. 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. It is suggested that melittin prepares phospholipids for enzymatic activity of phospholipase by its reduced surface tension. The main biological function of phospholipase A2 is listed in the table below.

Adverse effects: is the major allergen of bee venom that can cause anaphylactic shock, at high concentrations, exposure to bee venom group III sPLA2 can result in damage to cellular membranes and necrotic cell death.
Therapeutic effects: anti-inflammatory effects (Promote Treg differentiation, suppress airway inflammation, protect cisplatin-induced renal inflammation, protect acetaminophen-induced liver inflammation), anti-neuronal injury and anti-nociceptive effects (reduce oxaliplatin-induced neuropathic pain), anti-tumor effects (inhibit growth of various cancer cell), vaccination approaches, anti-parasite, and anti-bacterial effects.

Biological and therapeutic effect of phospholipase A2: it destroys phospholipids and dissolves the cell membrane of blood bodies; lowers the blood coagulation and blood pressure, prevents neuronal cell death caused by prion peptides. FLA2 has an immunomodulatory effect, acts against neurodegenerative diseases such as Parkinson, MS, Alzheimers. Additionally, it acts against different inflammatory diseases,

including lupus nephritis, cisplatin-induced nephrotoxicity, hepato toxicity and allergic asthma, it may as well act against acute lung inflammation induced by radiotherapy. FLA2 has additionally antinociceptive, anticancer, anti-bacterial, anti-parasitic and immunotherapeutic effects. However, it induces inflammation, because it is the strongest allergen and thus the most harmful bee venom component. FLA2 toxicity measured in rat experiments is 7.5 mg/kg.

Mast Cell Degranulating (MCD)

Mast Cell Degranulating (MCD**)** peptide is a cationic 22-amino acid residue peptide. At low concentrations, MCD peptide can stimulate mast cell degranulation. At higher concentrations, it has anti-inflammatory properties. In addition, it is a potential blocker of voltage-sensitive potassium channels. MCD peptide has immunotoxic as well as neurotoxic properties due to different active sites of the MCD peptide. The MCD peptide has an immunotoxic effect on mast cells by releasing histamine from these cells. MCD peptide has also been described as a potent modulator of voltage-gated ionic channels. It binds to several subclasses of voltage-gated potassium channels (Kv channels), including Kv1.1, Kv1.6, and less potently to Kv1.2. Accordingly, MCD peptide can act in various regions of rat brain, including cerebellum, brainstem, hypothalamus, striatum, midbrain, cortex and hippocampus. The neurotoxicity of MCD peptide is distincted from its histamine releasing function.

The histamine releasing function of MCD peptide, at low concentrations, causes the degranulation of mast cell and shows anti-inflammatory activity at higher concentrations. These actions of MCD peptide on mast cells is thought to be involved in allergic and inflammatory processes related to type I hypersensitivity reaction. MCD peptide shows neurotoxicity by inducing epileptiform seizures in rat, when intraventricularly injected. This toxicity is caused by the blockage of voltage-gated potassium channels. However, there is no toxicity of MCD administered peripherally, even at high doses. As a mast cell activator, the MCD peptide evokes large increases in antigen-specific serum immunoglobulin G (IgG) responses. Therefore, it is used as a vaccine adjuvant. MCD peptide analogs, such as [Ala12] MCD, provide a base for designing agents that can prevent IgE/Fc-RIa interactions and reduce allergic conditions.

Biological and therapeutic effect of MCD: lyses mast cells, releasing histamine, serotonine and heparine, melittin-like effect increasing capillary permeability; anti-inflammatory, simulates the central nervous system. MCD has relatively low toxicity, the toxicity measured in rat experiments is 40 mg/kg.

Bee Venom Collection

Early collection methods required surgical removal of the venom gland or squeezing each individual bee until a droplet could be collected from the tip of the sting. Now

standard procedure is extraction by the electro-shock method. Different extraction or collection methods result in different compositions of the final products.

Photo 11. Bee venom collectors

Venom collected from surgically removed venom sacs showed different protein contents from that collected with the 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 to preserve more of the volatile compounds. The various trap designs stimulate bees by applying a mild electric shock through wires above the collecting tray. The most widely used designs are modifications of the one first presented by Benton *et al*., (1963). The trays are placed either.

Between the bottom board and brood chamber at the hive entrance or in a special box between supers and the hive cover. When shocked, bees sting the surface on which they are walking. In some traps, this may be a glass plate or a thin (0.13 mm thick) plastic membrane, nylon taffeta or silicon rubber under which a collecting plate (preferably made of

glass) or absorbent tissue receives the venom. Venom dries rapidly on glass plates and can be scraped off with a razor blade or knife. Absorbent tissue is washed in distilled water to extract the venom, which then should be freeze-dried. Collection on glass is generally easier and produces a product which is easier to store, ship and process. It is unlikely that a bee will eject all the contents of its venom sac, even after repeated stinging. Therefore, typically, only 0.5 to 1.0 jil of venom can be collected per bee, with an average of ten stings per bee. This results in less than 0.1 ijg of dry venom per bee. Consequently, at least 1 million stings are required to make onegram of dry bee venom. Instead of collecting bee venom, adult bees may be used to sting the patient directly. This is the way to apply the venom in its freshest, most complete, and cheapest form. To collect the bees, a small hole is made in the brood chamber, super or inner cover. To avoid colony disturbance, the hole is opened, and a collecting jar placed over it until enough bees have come out. Small groups (10-100) of workers can be maintained at home for up to 2 weeks. They should be kept in the dark, in a small box (with one side made of flyscreen) and with access to sugar syrup. Alternatively, bees can be collected from frames or the hive entrance by a suction device. However, a screen should be placed over the tube leading to the mouthpiece to prevent any bees from reaching the mouth.

Efficacy and Safety of Bee Venom Treatment

Photo 12. Stinging bee

During the last seven decades, over 1700 scientific publications on the composition and various effects of bee venom in animals and humans have been published. An overwhelming proportion comes from Eastern Europe and Asia. Most of them concentrate on demonstrating the site specific, physiological effects of individual components such as membrane destruction, toxicity, or the stimulation or blocking of enzyme reactions. This has largely increased our understanding of the processes occurring after a sting, the physiological effects of isolated venom compounds and the substances responsible for most of the allergic reactions. It has contributed little to verifying the increasing claims of different therapeutic values attributed to honeybee venom, however. A study with whole bee venom on dogs (Vick and Brooks, 1972) and rats (Dunn, 1984) showed that melittin and apamine produce increased plasma cortisol. Together with various other arguments, this suggests that many of the curative effects of bee venom may work through stimulation of the body's enzyme and immune system, in a way like the common drug cortisone. Cortisone has been used in the treatment of many ailments, but it is also known to have strong, undesirable side-effects.

Melittin also appears to have toxic side effects as do some of the other individual compounds in venom. When whole venom is applied however, no side-effects have been shown, other than in allergic patients (Broadman, 1962 and Weeks, 1992 personal communication). The anti-inflammatory effects of bee venom are perhaps the best studied and the various mechanisms have been repeatedly described in scientific literature (Rekkaand Kourounakis, 1990; Kim, 1989 and others). The neurotoxic venom compounds have shown a potential benefit for epileptic patients (Ziai, 1990). The protective value of bee venom and melittin against the lethal or damaging effects of x-rays has been investigated (Shipman and Cole, 1967 and Ginsberg *et al*., 1968). Though these and many other results are encouraging, no clinical studies have been carried out to verify the effectiveness using tests accepted by the Western medical establishment. Nevertheless, more and more physicians and healers are experimenting with this benign treatment after they have tested the patient's allergic reactions to bee venom. Recently, after long efforts by the American Apitherapy Society and its members, some interest has been shown by national institutions in several Western European countries and the USA for clinical and large-scale tests of bee venom therapy. Good summary of the scientific studies, with further references can be found in Banks and Shipolini (1986) and Schmidt (1992). Summaries of some of the major specific effects of the various venom compounds that are shorter and more easily understood, can be found in Mraz (1983), Dotimas and Hider (1987), Crane (1990) and Schmidt

and Buchmann (1992). The American Apitherapy Society keeps records of scientific as well as anecdotal information on the use of bee venom. It is also probably the best source of information on any subject related to apitherapy. Bee venom therapy (BVT) is the therapeutic application of bee venom into the body for the treatment of diseases, which has been used in oriental traditional medicine since 1000-3000 BC. BVT was practiced by ancient Egyptians, Chinese, and Greek therapists, including Hippocrates.The use of BVT is since beekeepers (who often get stung) are very rarely suffering from arthritis or troubles with their joints and muscles. BVT has firstly been widely used for treating inflammatory diseases and pain diseases in oriental traditional medicine. In Western European and North American countries legally accepted medical use of bee venom is for desensitizing people who are allergic (hypersensitive) and in the Republic of China, bee venom therapy is combined with acupuncture.

Bee venom acupuncture is a form of acupuncture in which bee venom is applied to the tips of acupuncture needles, stingers are extracted from bees, or bees are held with an instrument exposing the stinger and applied to acupoints on the skin. It has been used in man to successfully treat a number of musculoskeletal diseases such as lumbar disc disease, osteoarthritis of the knee, rheumatoid arthritis, adhesive capsulitis, and lateral epicondylitis Injection of bee venom can also alleviate neurological conditions, including peripheral neuropathies, stroke and Parkinson's disease. It has even been

piloted in one series to alleviate depression. The potential of bee venom acupuncture to treat disease in man include musculoskeletal pain, neuropathic pain, neuropsychiatric disorders as well as autoimmune disorders (rheumatoid arthritis).

Bee venom has long been used in traditional medicine for the treatment of various kinds of rheumatism. Although venoms of the different honeybee species differ slightly, there have been reports of successful rheumatism treatment with*Apis dorsata*venom by Sharma and Singh (1983) and with*A. cerana*venom by Krell (1992, unpublished). The list of benefits to human beings as well as to animals is very long. Most of the reports of cures are of individual cases, though several unrelated patients have experienced the improvement or cure of similar ailments. Bee venom treatments are often accompanied by changes in lifestyle, nutrition or other which may account for part, if not most of the benefits from treatments. Reported clinical tests were often conducted in countries with less rigorous methods than the standard Western, double-blind placebo tests. Despite these considerations, many patients did report positive results and many of the successful treatments occurred after established medical or surgical procedures had failed. However, there is a very real resistance in Western medical circles either to accept these results or to test bee venom treatments according to Western medical standards. The diseases and problems which have been reported by patients or doctors as improved or healed with bee venom therapy are listed below.

Humans		
arthritis	multiple sclerosis	premenstrual syndrome
epilepsy	Bursitis	ligaments injuries
mastis	Cancer	sore throat
chronic pain	Migraine	general immuno-stimulant
dereases blood viscosity and coagulability	dilates capillaries and arteries	decreases blood cholesterol level
neruoses	Rhinosinusitis	endoarteriosis
therosclerosis	Polyneuritis	radicultitis
infectious spondylitis	Neuralgia	endoarthritis
infect. polyarthritis	Malaria	intercostal myalgia
myositis	tropical ulcers	slowly healing wounds
thrombophiletritis	cancer, temporary	keratoconjunctivitis
iritis	Iridocytis	asthma

Table. 10. List of diseases and health problems improved or healed according to anecdotal reports

This does not constituent an endorsement or recommendation for the treatments. Stinging should never be tried unless there is immediate access to emergency treatment in case of an allergic reaction. **Side effects of bee venom acupuncture in man include** an aphylactic shock, guillaume-rare syndrome, an irreversible

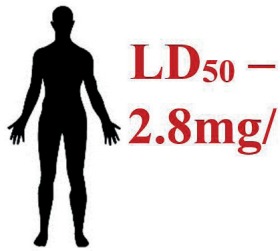

Fig. 23. The median lethal dose (LD 50) of bee venom for an adult human is 2,8 mg /kg of body weight.

ulnar nerve injury, thrombocytopenia with ecchymoses, an "acute lung injury", arrhythmia, stroke, nephrotic syndrome, pulmonary edema, liver failure, hepatitis, uterine contractions, or dermatologic complications. The bee venom is safe for human treatments; the median lethal dose (LD_{50}) for an adult human is 2.8 mg /kg of body weight. As an example, a person weighing 60 kg has a 50% chance of surviving after injections 168 mg of bee venom. 560 stings could be lethal for such a person if 0.3 mg of venom is injected per sting. For a child weighing 10 kg only 90 stings could be deadly. It is necessary, before bee venom use for therapeutic purposes, to take all measures, including allergy testing to protect the patient and use correct dosage.

Bee Venom Products

Bee venom may be sold as whole bee extract, pure liquid venom, or an injectable solution, but in either form the market is extremely limited. Most venom is sold in a dry crystalline form. Since venom does not need to be processed, it can be prepared wherever bee venom therapy finds sufficient support. Production in small quantities is easy if stringent sanitary controls and aseptic working conditions are provided. For injections, the venom can be mixed at the time of injection with injectable fluids, such as distilled (sterile) water, saline solutions, and certain oils, or it may be taken from prepared ampoules. Ampoules with set doses of ready-to-inject venom should only be prepared by certified pharmaceutical laboratories, because of the need to maintain stringent

aseptic conditions and to measure the dosages very precisely. There are creams available which include bee venom (e.g. Forapin and Apicosan in Germany, Apivene in France, and Immenin in Austria) which are used for external application on arthritic joints. Ointments can be prepared by thoroughly homogenizing bee venom with white Vaseline, petrolatum or melted animal fat, and salicylic acid, in the ratio of 1:10:1. The salicylic acid softens the skin, increases its permeability and is a treatment for rheumatism even on its own. The ointment may contain a small amount of silicate crystals to act as an abrasive. Other preparations consist of mixing bee venom with sterile, injectable fluids and packaging them in single dosages in glass vials or syringes. In some packages the dry venom is kept separate from the fluid and the two are mixed when the vial is broken. Some specialized laboratories may be able to separate and purify different venom compounds and sell them to scientific and pharmaceutical laboratories. Phospholipase $A_2$and highly active peptides are among some of the proteins purified from bee venom for scientific suppliers or laboratories. Entry to this limited market requires a highly sophisticated laboratory and very well-trained technicians and chemists. Bee venom is applied in skincare as well - it is considered a natural alternative to botox. The venom stimulates facial muscles for a natural anti-aging effect by smoothing and firming out fine lines and wrinkles. Melittin, present in bee venom, causes increased blood circulations, which means that the inner layers of the skin increase the production of elastin and collagen.

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 meet the wire electrodes will receive a small electrical shock. This causes bees to sting the glass, releasing the venom without losing their barbed sting. 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. 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 to preserve more of the volatile compounds. 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. 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. 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.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 **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, if strict hygiene controls and sterile working conditions can be provided. Bee venom **is sensitive to strong acids and bases as well as oxidizing agents and ethyl alcohol**. 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, if strict hygiene controls and sterile working conditions can be provided. 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.

Check Yourself

1. **Bee venom is synthesized in the venom glands of:**
   a) only queen
   b) workers and queen
   c) only drones
   d) it's not synthetized in venom glands.

2. **Most types of venom induce immediate pain because they contain:**
   a) phospholipases
   b) hyaluronidase
   c) all answers are correct.
   d) other enzymes

3. **Melittin - the main bee venom component:**
   a) has just a few positive biological effects and relatively high toxicity
   b) has many positive biological effects and very high toxicity.
   c) has many positive biological effects and relatively low toxicity.
   d) has no positive biological effects and relatively high toxicity.

4. Biological functions of melittin are:

a) hemolytic activity
b) anti-inflammatory activity
c) anticancer, antibacterial, antifungal, antiviral activities
d) all answers are correct.

5. The toxicity of melittin measured in rat experiments is:

a) 4 mg/kg b.w.
b) 40 mg/kg b.w.
c) 0,4 g/kg b.w.
d) 0,4 mg/kg b.w.

6. The only one that passes the blood-brain barrier is:

a) apamin
b) melittin.
c) adolapin
d) phospholipase

7. Apamin

a) inhibits small-conductance Na2+ activated K+ channels (SK channels) in neurons.
b) inhibits small-conductance Ca2+ activated K+ channels (SK channels) in neurons.
c) inhibits small-conductance Ca2+ activated Na+ channels (SK channels) in neurons.
d) inhibits small-conductance Ca2+ activated P- channels (SK channels) in neurons.

8. The polypeptide with 103 amino acid residues, and comprising 1% of dry matter bee venom molecular mass proved to be 11500 - 11092 Da. Apitoxin exhibited a potent analgetic effect is:

a) apamin
b) melittin
c) phospholipase
d) adolapin

9. One of the forms of bee venom therapy is acupuncture. It can be successfully used in treatment of:

a) lumbar disc disease
b) all answers are correct.
c) lumbar disc disease
d) rheumatoid arthritis

10. The bee venom median lethal dose (LD50) for an adult human

a) 2,8 mg/kg b.w.
b) 4,0 mg/kg b.w.
c) 8,5 mg/kg b.w.
d) 12,3 mg/kg b.w.e)

Answers: 1b, 2c, 3c, 4d, 5a, 6a, 7b, 8d, 9b, 10a

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Royal Jelly

Prof. Dr. Murat YILMAZ
Alkan ÇAĞLI *Selda MANAV*
Aydın Adnan Menderes University - Aydın Türkiye

Royal jelly, which is an important bee product in terms of apitherapy, was named "Royal Jelly" in 1793, which means perfect food in English. The use of royal jelly as a functional food within the scope of apitherapy started in the 1960s. In terms of the content and biological activity of royal jelly, it is used in many sectors from the pharmaceutical sector to cosmetics.

Photo 13. Royal jelly and bee larvae in honeycomb cells (https://en. wikipedia.org/ wiki/Royal_jelly)

Royal jelly, which is rich in nutrients, is a food substance secreted from the upper jaw (mandibular) and lateral pharynx (hypophryngeal) glands of 5-15 days old

worker bees. This foodstuff, which has a cream color, jelly consistency, distinctive odor and slightly burning taste, is used for feeding queen bees and young larvae. The content of royal jelly varies according to the nutrition of the bees, their age, the season and the age of the larvae. While all bee larvae are fed royal jelly only in the first three days, the larvae that will become queen bees are fed only with royal jelly throughout their larval and adult stages. Bee milk; As a result of the digestion of pollen and nectar in the digestive organs of young worker bees is secreted from the glands (mandibular and hypopharyngeal glands) in their heads. As soon as royal jelly is secreted and given to the oral cavity, it has the consistency of milk. As the effects of the benefits it provides in the colony on humans are revealed, the efforts to increase the production of royal jelly have accelerated, and more and more beekeepers in many countries have turned to royal jelly production. There is no official data on the international market for royal jelly in the world. In China, royal jelly has become a second product along with honey in the beekeeping industry. China ranks first in the world as the largest producer of royal jelly. The estimated annual royal jelly production of China is reported between 400-2000 tons in different sources, and China produces approximately 90% of the world's royal jelly production. When Türkiye is evaluated in terms of beekeeping, it ranks second after Turkey and China with its 8 million colony assets. Turkey ranks second after China with 114 thousand tons of honey production.

Photo 14. Royal jelly collecting.
Source: https://www.maybir.org.tr/ari-sutu-uretim-projesi.html

The structure and properties of royal jelly

Royal jelly is a water-soluble, viscous, gel-like substance with a density of 1.1 g/ml and a pH of 3.4-4.5. Its color is yellowish, and the color darkens as the storage time increases. The odor is sharp, the taste is sour or sweet. These are important sensory properties of royal jelly and are important quality criteria in this sense. **Royal jelly is affected by sunlight, humidity, heat and air very quickly and may lose its properties. For optimum quality of royal jelly, this product should be stored frozen.** The viscosity of royal jelly varies depending on the water content and the age of the bee, and its viscosity increases when stored at room temperature or in the refrigerator at +5 degrees. These changes result from ongoing enzymatic activities and interactions between lipid and protein fractions. In this respect, there is no international

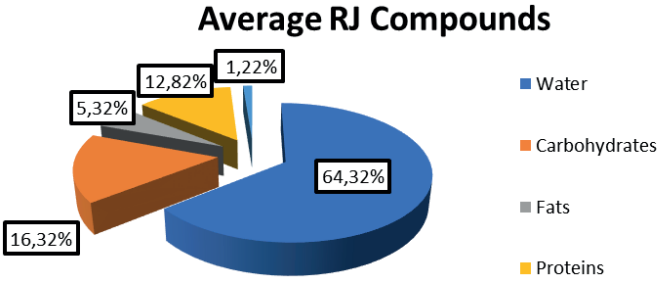
standard for royal jelly, but some countries have set standards for royal jelly. Some countries such as Switzerland, Bulgaria, Brazil and Uruguay have set national standards for this product. It is known that the International Honey Commission is working on developing an international standard in this regard. In the studies, the most important quality criterion for the standardization of royal jelly is 10-Hydroxy-2-Decenoic Acid (HDA). The 10-HDA content of royal jelly decreases with storage. This decrease is higher in honey containing royal jelly. The chemical structure of royal jelly can vary significantly depending on the season, region, race and nutritional status of the colonies used in the production of royal jelly.

Table 11. Components of fresh and frozen Royal Jelly (RJ)

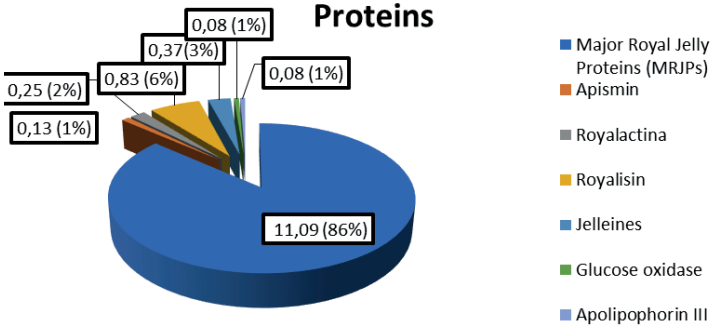
Components	Fresh RJ	Frozen RJ
Water (g/100g)	60-70	<5
Lipids (g/100g)	3-8	8-19
10-HDA (g/100g)	>1,4	>3,5
Protein (g/100g)	9-18	27-41
Fructose (g/100g)	3-13	-
Glucose (g/100g)	4-8	-
Sucrose (g/100g)	0,5-2,0	-
Ash (g/100g)	0,8-3,0	2-5
pH	3,4-4,5	3,4-4,5
Acidity (ml 0,1N NaOH/g)	3.0-6.0	
Furosine (mg/100 g protein)	<50	-

Table 11. (Bogdanov,2012; Ramadan ve Al-Ghamdi, 2012)

Arı sütü % 60-70 oranında su içerir. Kalan Kuru madde içeriği karbonhidratlar, proteinler, amino asitler ve yağdan oluşur. Az miktarda mineral ve vitamin de mevcuttur.



Graph 1. Average RJ Compounds (Fratini et.al., 2016)



Graph 2. 12,82% of the RJ compounds in average is made up of proteins: (Main RJ Proteins /Apalbumines (Fratini et.al., 2016).

Lipids

Despite their change due to fresh or frozen forms, lipids, holding 3%-219% of the dry weight of RJ, come in the second rank after proteins. 80 or 90 % of lipid fraction is composed of free fatty acids. Unlike so many animal and plant materials, fat acids of RJ are with 8-10 carbon atoms and either in hydroxyl fatty acids or in dicarboxylic acids form. These fatty acids are responsible for plenty of reported biological characteristics of RJ. The main acid is 10 hydroxy-2- decenoic acid and is seen at approximately 1, 9% rate. Its saturated equivalent, 10-hydroxy decenoic acid, follows it. In addition to free fatty acids, lipid fractions contain some neutral lipids, sterols (including cholesterol), and the unsaponifiable fractions of hydrocarbons similar to bee-wax extracts. Some of the fatty acids in RJ were determined to have antibacterial properties (Nagai and Inoue, 2005; Terada et al., 2011; Fratini et al. 2016). 10-HAD has also proved to have a significant biological role in the development of colony strategies (Wu et al. 1991). Apart from that, 10-HAD content has been adopted as an indicator for the quality and freshness analyses of RJ (Ferioli et al. 2007). Octanoic acid, which is less than 10-HAD, has been determined in the recent studies to defend the Queen Bee against The parasitic mite Varroa in addition to its nutrition function (Nazzi et al. 2009).

Minerals

Minerals and the other elements make up approximately 4-8 % of the dry substance of RJ. Main elements are K, P,

S, Na, Ca, Al, Mg, Zn, Fe, Cu and Mn; however, in tiny amounts (0, 01-1 mg/100 g), Ni, Cr, Sn, W, Sb, Ti and Bi also exists (Li and Chen, 2003; Ramadan and Al-Ghamdi, 2012). The existence of the minerals is related with the source of the nutrition, the production period, the environment and the biological factors of the bees and therefore can display variability (Sabatini et al. 2009). In addition, RJ has been determined to contain heterocyclic matters and a few small compounds classified under various chemical categories such as biopterin and neopterin (Bogdanov, 2012). Apart from these, low amounts of free nucleotides (adenosine, uridine, guanosine uridine and cytidine), phosphates, ATP, ADP, AMP, acetylcholine and gluconic, benzoic, malic, citric and lactic acids in RJ Sabatini et al. 2009; Bogdanov, 2012). However, the functions of all of these determined compounds are still ambiguous.

Vitamins

RJ is quite rich in vitamins. The vitamins it contains are riboflavin, thiamin, niacin, folic acid, pyridoxine, biotin, pantothenic acid and inositol and a little vitamin C. The vitamin content of RJ is subjected to seasonal changes as the variation of the pollens of the flowers that the worker bees pick, as the vitamin source fundamentally comes from the pollen (Biondi et al. 2003; Sabatini et al. 2009). RJ is generally rich in B group vitamins, particularly B1, B2, B6, B8, B9 and B12 (Viuda-Martos et al. 2008; Li et al. 2012). RJ doesn't contain

vitamins that melt in fat, such as A, D, E and K (Morita et al. 2012; Ramadan and Al-Ghamdi, 2012).

The Importance of Royal Jelly for Apitherapy

Royal jelly is used in many areas for humans. It has been used in cosmetics, stimulating physical performance, providing learning capacity and self-confidence, increasing resistance to sexual problems, anemia, cholesterol, viral infections, cancer, high and low blood pressure, atherosclerosis, chronic and recurrent diseases. Numerous studies have been conducted on laboratory animals on the effects of bee products and especially royal jelly, but not enough studies have been conducted on humans. However, many positive effects of royal jelly on living things are known. It has been reported that royal jelly has a positive effect on the cardiovascular system and regulates blood pressure. It has been reported that regular use for 2-3 weeks as an alternative medicine for anemia positively affects the quality and number of red blood cells, and can be used in the treatment of hypertension and atherosclerosis. In some studies, trans-2-octenic acid and hydroxydecanoic acid in royal jelly may be responsible for the anti-hypertensive effect, and royal jelly has been associated with protective and therapeutic effects in cases of adrenaline-induced arrhythmia (irregularity in heartbeat), but it still has no effect on heart rate. effect has not been fully observed. Elderly people were given 10 g of royal jelly daily for 14 days orally, the ratio of good cholesterol (HDL) in the blood increased and the ratio of

bad cholesterol (LDL) decreased. In another study, when 6 g royal jelly / day was given orally for 4 weeks, a decrease was observed in the total LDL cholesterol ratio in the blood, but it did not affect the good cholesterol ratio (HDL) and triglyceride ratios. In studies conducted with humans and experimental animals, it has been observed that royal jelly taken orally has a positive effect on cholesterol and triglyceride levels in terms of health and reduces bad cholesterol levels. Recently, various studies have been carried out on the antimicrobial activity of this valuable bee product, since royal jelly is seen as a product that can be used in the field of medicine as well as its widespread traditional use due to its protein and lipid components. It has been reported that royalicin, 10-hydroxy-2-decenoic acid, gelleins, major royal jelly proteins in unprocessed royal jelly have antimicrobial activity against different bacteria. Royal jelly and other natural bee products have shown antimicrobial activities in various areas where they are used as natural additives. Storage conditions of royal jelly are important for human use. **Royal jelly is sensitive to light and heat and undergoes oxidation in direct contact with air**. Expected benefits cannot be obtained from royal jelly that is not collected and stored under appropriate conditions.

It has been reported that 10-hydroxy-2-decenoic acid has antibiotic activity against some bacteria and fungi (Micrococcus pyogenes, Escherichia coli, Neurospora sitophila). One of the most important quality factors in royal jelly is the amount of 10-hydroxy-2-decenoic acid (10 HDA),

and it should be at least 1.40% and above by mass in royal jelly produced under suitable conditions. Studies have shown that royal jelly and the 10-HDA it contains are effective against many bacteria, including Escherichia coli and Micrococcus pyogenes.

In 1990, Fujiwara et al., isolated and purified Royalisin from royal jelly MIC (Minimum Inhibitory Concentration) evaluation, was tested on both Gram positive and Gram negative bacteria of raw royal jelly and showed that these bacteria have a low resistance to Royalisin. . In addition, Royalisin, Bacillus subtilis and Paenibacillus larvae subsp. While it is effective against Micrococcus luteus (Sarcina lutea), its effect has not been determined. Some researchers have determined that royal jelly has antibacterial effect against Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli. In 2013, Moselhy et al. reported that gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) were more sensitive to any sample of royal jelly compared to Gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli). The bactericidal or bacteriostatic effect of royal jelly is closely related to geographical origin, related botanical species and genetic variability between colonies. It has been stated that royal jelly is effective against infection of skin wounds and some bacteria. It has been reported that royal jelly has an insulin-like effect and is used to protect against diabetes and lower blood sugar levels, especially in China and Japan. Peptides very similar to mammalian insulin have

been found in royal jelly. In some studies, the effects of royal jelly were examined in children with leukemia, lymphoma and hepatoblastoma, and in these patients; Improvement in general condition, weight gain, increased leukocyte, lymphocyte and neutrophil levels in the blood have been reported. It has been stated in some studies that royal jelly is effective on breast cancer. It has also been reported to be effective on ulcer and adrenal gland cancer. It has been reported that royal jelly contains amino acids and gamma globulin, unsaturated fatty acids, hormones, enzymes, proteins, vitamins E and A, which help the immune system to fight against infections.

The effect of royal jelly on reproduction and fertility in honey bees is known. It is known that royal jelly positively affects reproduction and fertility in studies conducted in humans and some other living things. It has been reported that royal jelly increases ovulation and sperm quality in men and women, improves fertility by providing hormonal balance, and affects the person positively, especially in cases of low libido and impotence in the elderly. In terms of pharmacological effects, royal jelly has been reported to have estrogen hormone activity, but this effect is effective at low levels. In the 1970s, some studies were conducted on experimental animals using royal jelly to reduce the effects of menopause in women, but sufficient clinical studies on humans were not conducted. In the study on male mice, the effect of royal jelly on spermatozoa density, spermatozoa motility and abnormal spermatozoa ratio was found to be significant.

The use of royal jelly increases the success rate of in vitro fertilization methods. Human studies have reported that royal jelly has a beneficial effect on sperm count and motility and improves the fertilization ability of male gametes.

There is an increase in fertility in women who consume royal jelly regularly for at least 6 months, the most important reason for this increase is that royal jelly is an important source of para-amino benzoic acid, and Pantothenic acid (vitamin B5) it contains together with this acid has a beneficial effect on healthy hair and skin. has a positive effect. The application of royal jelly in farm animals (chicken, rabbit, buffalo and sheep) contributed to the improvement of pregnancy and birth rates. It has been proven that the combination of royal jelly with 12-day progesterone applications in creating estrus in sheep gives successful results. In recent years, royal jelly has been used as a natural alternative to synthetic hormones to improve the reproductive efficiency of sheep and to solve reproductive problems. It is reported that the use of royal jelly positively affects estrus synchronization and pregnancy rate in sheep. In different studies conducted in sheep, positive results were obtained with intravaginal administration of royal jelly and progesterone and improved pregnancy rates. In addition to the positive effect of royal jelly on the synchronization of estrus, pregnancy and fertility, it has similar effects to the chorionic gonadotropin hormone. However, oral administration of royal jelly was not effective in improving oestrus in sheep during the transition between inactive and active breeding seasons.

In a study in mice, it was noted that royal jelly had a positive effect against osteoporosis. As a result of increased intestinal absorption of calcium, an increase in bone calcium levels and an improvement in bone mass have been observed.

Since ancient times, it has been believed that royal jelly can prolong human life, as it is known that the queen bee fed with royal jelly has a much longer life expectancy than worker bees. Despite much talk about the anti-aging potential of this product, few clinical studies have been conducted on this effect of royal jelly. Studies have shown that royal jelly protects DNA against oxidative damage. It has been shown to reduce oxidative stress and prolong lifespan in mice fed royal jelly.

In animal experiments and studies on humans, it has been observed that royal jelly causes an acceleration in metabolism. After the studies that concluded that royal jelly has an immunoregulatory effect, the effects of this effect on cancer, allergy and inflammation were investigated. There are studies showing that it may have anti-inflammatory and anti-allergic effects, and it has been suggested that it may have anti-aging effects in humans with its anti-inflammatory effect mechanism. It has been concluded in some studies that it has a stimulating effect on the central nervous system, has neuroprotective, neurotrophic effects and directly affects brain cell differentiation. These findings brought up the issue that royal jelly can be used to prevent neuronal loss in diseases such as Parkinson's and Alzheimer's and to increase neurogenesis. It is known that royal jelly reduces blood plasma levels of cholesterol and

triglycerides, as a result of studies conducted in animals against diseases seen in humans. Royal jelly has no effect on blood plasma lipid levels in rabbits, and it has been reported that the cholesterol content in the blood of animals fed with a diet that causes blood cholesterol levels may decrease. In addition, royal jelly promotes bone healing in rabbits, accelerates the healing of skin lesions and has an anti-inflammatory effect in rats.

The cardioprotective action mechanisms of royal jelly shown in animal experiments are as follows; There are studies showing that there is a decrease in serum cholesterol and triglyceride levels, an increase in HDL levels, a decrease in LDL levels, a decrease in plasma fibrinogen level and thrombosis, Antihypertensive effect, Antioxidative, protective against the effects of radiation and liver protective effects of royal jelly. It was concluded that it stimulated bone formation and accelerated bone healing in rabbits and prevented osteoporosis in mice. In mouse experiments, it has been shown to prevent the formation of atopic dermatitis-like skin lesions. It has been concluded that it supports collagen production in in-vitro experiments performed in cell cultures.

RJ PRODUCTION

Natural Production Method

In the RJ production in a natural way, it can be taken out by distorting Queen Bee cells in the honeycombs (swarm) with larvae during the routine controls in April-August period. The

Queen Bee in a strong hive is taken out and honeycombs with 1-2 eggs and with 1 day larvae from other hives are inserted into this hive. In this way, Queen Bee cells production is egged by the worker bees. After this process, the maximum yield is provided from the cells containing three days old queen bee larvae. This operation goes on 20-30 days with this production technique. Later on, the Queen Bee is returned to the hive again and the colony is turned to its previous situation. With this natural production method, 2-25 g RJ can be taken from one hive (Serefoglu, 2009).

Production through grafting:

RJ production is closely related with Queen Bee production. In order to produce RJ, Queen Bee cells are prepared artificially and 1-1, 5 days old larvae are transferred into these cells. Worker bees secrete RJ into these cells to feed these larvae. Without letting the larvae consume RJ, the hives are opened 24-36 hours later and the frames are taken out and the larvae in the cells are taken out with special needles and the RJ in the cells is compiled. Before a larva reaches to the 3rd form from above, it must be taken out from the cell and RJ must be harvested. From a natural Queen Bee cell, or from an artificial one, it is possible to harvest 100-250 mg RJ in one day. In RJ harvest, timing is extremely significant because larvae consume RJ so fast. The preparations done for RJ production look like the ones done for Queen Bee Production.

RJ production takes place in four stages. These are:

1. **Production of the cells**: The cells that will be produced for larvae transfer could be from bee wax, or artificial queen bee cells made of plastic could be used for this purpose as well. In the RJ production done for particularly commercial profits, plastic queen bee cells are used more extensively. Main cells are made of pure bee wax with the help of a wooden block in 8-9 mm diameter, 10 mm deep and at least 1 mm thick. Bee wax is melted in a double walled melting pot. Queen bee cell block is dipped into the water in a container and then into the melted bee wax at 1 cm depth. In order to reach the desired thickness, this operation can be repeated a few times according to the temperature of the wax. The pre-melted wax is poured over the queen bee cell dipped into wax and is put on the lath prepared beforehand and with the melted wax, it is fixed on the lath. After waiting for some time, it is dipped into cold water and the block was pulled off, and so queen bee cell preparation is completed.
2. **The preparation of the starting colonies:** The Queen Bee of a two leveled powerful colony is taken to another hive together with a few frames with bees. The remaining bees are shaken into the incubation place and are forced into 6 or 8 frames. These bees are fed with syrup and cake every day. Two days

after the Queen Bee is taken out, the hive is opened and all the natural cells are removed and the frames in the hive are re-arranged in such a way that they should be in the following order; with honey-with pollen- with closed larvae- with open larvae- space- with pollen- with open larvae- with closed larvae- with honey.

3. **Grafting Larvae:** From a powerful hive in the hive a honeycomb carrying 12-24 hours old larvae on is taken to the transfer room. The larvae on the honeycomb are taken by means of a transfer spoon and left into the queen bee cells which have been prepared before by placing inside of each one drop of RJ- water mixture at 1/1 rate. The larvae transferred are given to the starting colony that has been prepared before by attaching the transfer frame. After 48-72 hours, the transfer frames in the starting colony are taken to the transfer room.
4. **Harvesting the RJ:** The larvae on the RJ are taken out with the help of a thin plastic or wooden spoon and the underside RJ is taken into dark glass jars. From one queen bee cell, approximately 148-281 mg RJ can be harvested


Photo 15. RJ collecting
https://www.gidahatti.com/ari-sutu-hasadi-basladi-103499/

The amount of the RJ to be collected depends on the starting colony and the transferred larvae genotype, on the nourishment of the starting colonies, on the number of the larvae transferred to the starting colony, on the ages of the larvae transferred, on the type of the nutrient given to the colony, on the power of the starting colony, on the number of the young worker bees in the starting colony and on the temperature and the moisture rate of the larvae transfer room. The chemical structure of the RJ could display variations at some rates due to the producer countries. Some genetic differences are the case among various bee breeds regarding RJ production (Serefoglu, 2009).

The amount of the RJ to be harvested:

The fundamental scale of the productivity in a bee breeding enterprise is the yield per colony during a production season. In general, the amount of the RJ produced in the colony changes depending on the number and the age of the feeding worker bees and on the number of the grafted larvae. As the number of the grafted larvae increases, the amount of

RJ in the cell falls; however, an increase happens in the total RJ the colony produces (Karacaoglu et al. 2004).

One of the significant concerns in RJ production is the technique used. In a study Sahinler and Kaftanoglu conducted (2005), it was determined that the grafting rate and RJ yield is higher in the early spring than in the summer, that the average grafting rate all thorough the season and the RJ yield per cell were higher in colonies without the Queen bee than in the ones with a Queen Bee, and that the average grafting rate in the colonies without a Queen Bee was 88, 2 % and RJ yield was 0.263 g and in the colonies with a Queen Bee was 72,1 and 0.214g respectively. In a study done on the RJ yields of various genotype bees (Mugla, Caucasian, and Carniolan), the yields were found as 0.325g, 0,200 g and 0.372 g respectively and the effect of bee genotype was found to be significant on RJ yield (Sahinler and Kaftanoglu, 2005). In the study conducted, the highest RJ yields were succeeded in April. For this reason, it can be said that the bee genotype, the regional conditions and the season are all significant factors in RJ yield.

The Preservation Conditions of RJ:

The RJ is affected by the temperature, light, moisture, air and so many other factors and for this reason the storage of it is difficult. It must be stored in dark colored glass jars at +4 ^{o} C in the refrigerator, and in addition, the RJ containers should be carried in private freezers when they are taken out off the fridge to be carried somewhere. RJ could be stored without

spoilage for 6 hours at room temperature, for 2 months at +5 °C in the refrigerator, and for 6 months as frozen or dried at –18 °C. It can also be stored or 24 months at –170 °C.

Photo 16. Freezing royal jelly

Freezing royal jelly

Cooling and freezing delays and reduces chemical changes in royal jelly during storage. The following points should be considered for the storage of fresh royal jelly.

(1) Transfer the royal jelly into a dark and airtight container immediately after collection.

If royal jelly is to be consumed quickly,

(2) Refrigerate at 0–5 °C.

Alternatively, if royal jelly is to be stored for a longer time,

(3) Freeze at temperatures below -18 °C.

- Royal jelly should be packed in dark containers to protect it from light.
- The container must be airtight to protect it from oxidation.
- Storage and shelf life should be as short as possible, as there are no criteria for establishing "safety" limits for product effectiveness.
- After thawing and packaging, the product should not be stored in the refrigerator for more than 12 months.
- Repeated freeze-thaw cycles should be avoided.

Apitherapeutic Use of Royal Jelly

Royal jelly is sold fresh, frozen, unprocessed except for chilling, mixed with other products or freeze-dried. Fresh production and sale do not require special technology. It is directly used in many foods and diets, such as cosmetics or medicinal products, in the unprocessed form. In large-scale industrial use, royal jelly is preferred as dry frozen form due to its ease of obtaining and storing. Dry-frozen royal jelly can contain some products as well as in fresh form. Be very careful with the wording in the advertisements and the suggestions on the labels on the packages. Scammers have a great danger in the long run rather than the short-term gain, such as over-inflated claims increasing the price of the product. Products containing royal jelly must be specially marked or packaged to distinguish them from similar products that do not contain royal jelly.

Royal jelly is also used as a product defined as dietary supplement. These are not products that are consumed either for pleasure or because of their calorie content. They are added to supplement the diet with substances that may be lacking in the diet. In fact, the use of royal jelly depends on its supposed therapeutic value and stimulating effect. It cannot be defined as a medicinal product, and the data required for its definition in this category are missing. If royal jelly is to be used as a medicinal product, it should depend on medical prescriptions and the production and marketing of products containing royal jelly should be in a special area of the pharmaceutical industry. Royal jelly is sold and consumed as it is harvested from the hive. It is preferred by many consumers in unprocessed and natural form. Because royal jelly does not require any special technology so that it does not lose its naturalness. The taste is actually not very pleasant. Its particular medicinal aspect is undervalued and royal jelly can be mixed with some honey, sugar syrup or water or encapsulated. Unprocessed royal jelly is usually packaged in small, dark glass bottles of 10, 15, 20 pieces in a box. It contains a small plastic spatula and appropriate doses of 250-500mg. Special isothermal packaging system is used to protect the product from possible temperature fluctuations. Sold in Italy in special glass syringes that provide significant protection against oxidation. Now, royal jelly and other bee bee products are processed and packaged in all pharmacies and sold commercially for apitherapy purposes, as food and drug supplements

Photo 17. Commercial RJ products

In addition, producers sell royal jelly as pure royal jelly in closed thimbles and in original queen cell thimbles, which are subsequently removed and discarded. The thimbles can be closed with queen bee thimbles prepared with liquid wax or the tip can be compressed. Queen bee thimbles prepared in this way are packed in small plastic boxes or glass jars with a small spatula. The disadvantage of this type of packaging is that royal jelly is not well preserved (two weeks in the refrigerator or a few weeks when frozen immediately) and is only sold directly from the producer to the consumer. On the other hand, such sales can be extremely lucrative and impressive so that consumers can be assured that they are buying unprocessed and fresh royal jelly. The net weight of the normal variation in the content of the queen bee thimble should be given as the smallest possible amount (for example, the minimum content of 250 mg/thimble). Royal jelly sold in the ways described should be kept at a temperature below 5°C during storage, transportation and retail sale.The mixture of honey and royal jelly (1-3% royal jelly) is the most common usage. The advantages of this product include that it does not require a special technology

and that honey does not make any visible changes in royal jelly. The resulting product is sweet and contains the beneficial effects of honey and royal jelly. One teaspoon of the mixture may contain 100-300 mg of royal jelly. This approximate dosage of royal jelly is the most general recommended use. There is not enough information about the storage method of royal jelly with this type of mixture. For this reason, it should be stored in the refrigerator. Another food enriched with royal jelly in some European countries is yogurt, which has a similar acidity to royal jelly. Mixtures made with yogurt should also be stored in the refrigerator. Yogurt is already a popular food for health-conscious consumers, besides being enriched with royal jelly. Sometimes vitamin supplements and juices are enriched with freeze-dried royal jelly. Royal jelly is widely used as a beverage in Asia. Royal jelly is also sold in gel made with honey, sugar, jam and pectin. However, there is not enough data to be used on the longevity or permanent effect of royal jelly in this way. The medicine-like product category is similar to drugs, depending on their form of presentation. However, more advanced technology and processes such as quality control are required for production and packaging. For the same reasons, dry frozen royal jelly is used in most of these applications. Unfortunately, the pricing of these products is not always reflected in the product quality. In drug-like formulations, royal jelly is mostly used for stimulating effects and solving specific health problems. Variation of the formulations can often be used, in part containing the anti-

anxiety compositions. The dosages to be used can be any of the following,

- Single-dose packaging of dry royal jelly with a separate solvent,
- Packaged as a single or multi-dose liquid for injection or oral use.
- Packaging of a dose in the form of a mixed composition, tablet or capsule, with or without solvent

Since a dose containing only 250 mg of dry frozen royal jelly will seem very small, products that will give a pleasant taste are used with substances such as sugar, salt, flavors, citric acid, glycine in order to increase the volume. Additional compounds such as plant extracts, yeasts, pollen extracts are usually mixed with royal jelly. Most of the time, the packages contain royal jelly and solvent liquid in dry condition in separate containers. This separation facilitates the storage life, transportation and marketing of royal jelly. Some packages contain royal jelly in dry phase in a special cover in which royal jelly powder is mixed into the solvent when the royal jelly is opened. In tablet form, powdered sugar and a binder such as gum arabic are generally used. Tablet making machines are required for further production. Hard and soft gelatin capsules can also be used with similar formulations. Hard capsules can be filled by hand in small scales or by machine at the more industrial level. But soft capsules and gelatin dragees require expensive equipment. Royal jelly is available in many dermatological preparations. However, it is mostly used for

skin rejuvenation and rejuvenation. It is also used in creams or ointments used on burns and other wounds. It is generally used in dosages from 0.05% to 1%. The competitiveness of the European beekeeping sector is gradually declining as the production of beekeepers decreases as a direct result of the decrease in the bee population; this means lower economies of scale, underutilized resources and higher relative production costs. In addition, beekeeping products produced in countries with much lower quality standards, sometimes adulterated with their equivalents and supplemented with sweetening products, gain market share in Europe due to unfair competition. There is a lack of existing standards at European (and international) level for certain bee products such as pollen and royal jelly. Few countries in Europe have some guidelines or regional standards for products other than honey, but broad standardization is lacking. https://cordis.europa.eu/project/id/243594

Allergic effect of royal jelly

Allergic reactions are the most common side effects of RJ. After the intake of RJ orally, it is possible to witness various side effects from simple ones such as allergic reactions, asthma and anaphylactic shock to serious cases like intestinal bleeding, gastrointestinal problems, atopies and even death (Thien et al. 1996). Even though no death cases have been reported for the patients who have taken RJ during a fit so far, it is recommended that Rj sould not be taken in these cases. People who have allergy particularly against bee products

such as pollen, honey and bee venom must not take RJ. If RJ is to be applied on the skin either directly or together with some various ointments, it could cause skin rash or eczema (Takahashi et al. 1983; Jeung et al. 1997; Yonei et al., 1997). It is strictly recommended that if RJ is thought to be used for various health problems, this must be done under the doctor supervision, according to convenient methods and at the correct doses.

Residual effects of major veterinary drugs and acaricides in royal jelly

Bee products can be contaminated by different sources of contamination, including environmental and beekeeping sources. The most important contaminants in royal jelly are veterinary drugs used against bee diseases or to prevent disease outbreaks. Acaricides used in varroa control are also important contaminants of bee products. Although most veterinary drugs are not authorized for the treatment of honey bees in the EU or are strictly limited in other countries, veterinary drug residues may be found in some royal jelly samples. The most important and harmful veterinary drug residues in royal jelly are chloramphenicol, nitroimidazole, sulfonamides, fluoroquinolones, macrolides and tetracyclines. Fluvalinate and amitraz are the main acaricides used in beekeeping and are usually retained in bee products. These chemicals may have negative effects on royal jelly quality, as well as adverse effects on human and animal health for apitherapy in the

use of royal jelly. There are methods for the determination of chemical residues in royal jelly**.** Chloramphenicol (CAP) is a broad-spectrum antibiotic with activity against various aerobic and anaerobic microorganisms. Its protein synthesis inhibitory properties have been used against various infectious diseases. It has been used to prevent foulbrood in beekeeping in Europe and America (Ortelli, Edder, & Corvi, Alıntı 2004). However, this drug has been found to have serious side effects such as aplastic anemia and hypersensitivity in humans (Allen, 1985), the European Community has banned the use of CAP in food-producing animals since 1994 to protect the health of consumers. As a result, CAP is listed in Group A of Council Directive 96/23/EC, including substances for which a "zero tolerance residue limit" has been established in edible tissues. However, this drug is still used illegally in livestock due to its availability and low cost. The content of royal jelly contaminants is relatively low compared to other bee products (Fleche et al., 1997). Recently, the problem of honey and RJ contamination by antibiotics has arisen. Although most studies are about residues in honey, the use of antibiotics in the colony can also contaminate royal jelly (Matsuka and Nakamura, 1990). For this reason, prohibited drugs, especially antibiotics, should not be used and attention should be paid to unnecessary drug use, drug effect periods and harvest periods. It is recommended that hives that will produce royal jelly for apitherapy should be controlled and supervised in this respect.

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Bee Pollen and Bee Bread

Dr. Anzelika DAUTARTE
Agriculture Academy of Vytautas Magnus University - Lithuania

An interest in substances of natural origin has been a subject that is increasing constantly-both those known for many years and recently discovered are of great interest. This interest also applies to bee products because of their extensive nutritional and therapeutic properties; these products are known and used for several thousand years, but only recently, they became the subject of sparse documented scientific research. Recently, there has been an increasing demand for natural products, particularly bee products. Bee bread and pollen, due to their nutritional and medicinal properties, are used for apitherapeutic purposes. These include about 200 different substances, such as free amino acids and vitamins. Particular attention should be attributed to unsaturated fatty acids such as linoleic, linolenic, and arachidonic, which are found in pollen and bee bread. Therefore, bee bread that is rich in beneficial ingredients has proved to fulfill these expectations. It constitutes a beneficial, biologically active nutrient, which can be used in the food industry. After a period

of fascination with highly processed products, the return to natural foods, whose nutritional value is confirmed by the results of scientific research, is currently observed around the world. Bee bread and pollen contain the nutrients well absorbed by humans. Thus, they allow in supplementing nutritional deficiencies, as well as a better adaptation of an organism to adverse environmental conditions, improving the physical and mental state. In conclusion, it can be stated that bee products are characterized by many beneficial biological properties that can be successfully used in food technology and medicine.

Introduction to Pollen

Pollen is often regarded as "the world's best food product" (Bobis *et al.*, 2010). Global production of the pollen is around 1500 tons per year. The largest producers are China, Australia, and Argentina (Estevinho, Afonso, & Feas, 2011). Pollen is the male seed of flowers. It is required for the fertilization of the plant. The tiny particles consist of 50/1,000-millimeter corpuscles, formed at the free end of the stamen in the heart of the blossom. Every variety of flowers in the universe puts forth a dusting of pollen. Many orchard fruits and agricultural food crops do, too. Pollen grains, depending on the plant species, differ in shape, color, size, and weight. The grain shapes are diverse: round, cylindrical, bell-shaped, triangular, or thorny. In the dry state, these are mostly spherical or spindle-shaped formations, and after swelling, they may

have a round, triangular, cylindrical, bell-shaped thorn-like cross-section (see figure below).

Photo 18. Italian bee (Apis mellifera ligustica) on the white sweet clover (Melilotus albus)(picture by Ivar Leidus)

Pollen grains consist of three substances: the inside of the cell is filled with living cytoplasm. The inner layer of the cell wall, the intine, consists mainly of cellulose and pectin (see figure below). The outer cell wall, the exine, consists mainly of sporopollenin, an N-free polymeric substance belonging to the terpenes. Its chemical formula is $C_{90}H_{130\text{-}158}O_{24\text{-}44}$. Their diameter ranges from 0.01 to 0.05 mm (Barene *et al.*, 2015). Their weight is equal to a dozen or several dozens of micrograms. Most pollens consist of single grains which are sometimes joined with two or more grains. The colour of pollen loads is sometimes variable and reflects the diversity of plant species from which the pollen is obtained (Deveza *et al.*, 2015). The colour is usually in various shades of yellow, grey-white, orange, reddish, greenish, blue. Some differences in pollen colour depend on whether it was collected from the already open thecae, or the bee cracked them. Bees usually collect pollen from the same plant, but they sometimes collect pollen from many different plant species. The pollen grains depend on the plant species; they differ in shape, colour, size, and weight.

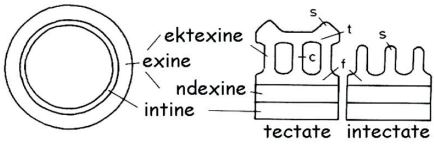

Fig. 24. Cross-section of the cell wall (structure) of a pollen grain (in: G. Lang, 1994, p. 44)

Photo 19. A honey bee gathering pollen (https://beewellholistichealth.com/buzz/2016/11/30/bee-pollen-as-food-and-medicine)

Bee pollen is a ball or pellet of field-gathered flower pollen packed by worker honeybees and used as the primary food source for the hive. It consists of simple sugars, protein, minerals and vitamins, fatty acids, and a small percentage of other components. Also called bee bread, or ambrosia, it is stored in brood cells, mixed with saliva, and sealed with a drop of honey. Bee pollen is harvested as food for humans, with various health claims, one of them being that the fermentation

process makes it much more potent than simple flower pollen. Bees collect pollen from plant anthers, mix it with a small dose of the secretion from salivary glands or nectar, and place it in specific baskets (called corbiculae) that are situated on the tibia of their hind legs – called pollen loads. Gathering pollen is not as easy as it sounds (figure below) (Fuenmayor *et al.*, 2014). Once a honeybee arrives at a flower, she settles herself in and nimbly scrapes off the powdery loose pollen from the stamen with her jaws and front legs, moistening it with a dab of the honey she brought with her from the hive. The enlarged and broadened tarsal segments of her legs have a thick trimming of bristles, called pollen combs. The bee uses these combs to brush the gold powder from her coat and legs in mid-flight. With a skilful pressing movement of her auricle, which is used as a hammer, she pushes the gathered gold into her baskets. Her pollen baskets, surrounded by a fringe of long hairs, are simply concave areas located on the outside of her tibias. When the bee's baskets are fully loaded, the microscopic golden dust has been tamped down into a single golden grain, or granule. After the pollen is collected, it's brought to the hive where it is packed in honeycomb cells. Then the surface of the collected pollen is covered with a thin layer of honey and wax, creating "bee bread." The bee bread undergoes anaerobic fermentation and is preserved by the arising lactic acid. The bee bread serves as the primary protein source for the bee colony. Pollen balls are stored in the chambers of honeybee hives, and sometimes in wood and mud created by female ground-nesting bees, such as the leaf-

cutting bee. With the leaf cutting bee, when the pollen ball is complete, a single female lays an egg on top of the pollen ball, and seals the brood cell. It differs from field gathered pollen as honeybee secretions induce a fermentation process, where biochemical transformations break down the walls of flower pollen grains and render the nutrients more readily available.

Photo 20. A pollen trap

Forager bees that gather pollen do not eat it themselves since they stop producing the proteolytic enzymes necessary to digest it when they transition to foraging. The foragers unload the pollen they gather directly into open cells located at the interface between the brood and stored honey, creating a typical band of what is called bee bread – the substance which is the main food source for honeybee larvae and workers. Foraging bees bring pollen back to the hive, where they pass it off to other worker bees, who pack the pollen into cells with their heads. During collection and possibly packing, the pollen is mixed with nectar and bee salivary secretions. Bee pollen is the primary source of protein for the hive. This method of packing can be seen in the bee species Arabian carpenter bee *(Xylocopa sulcatipes*and *X. varipuncta)*. Honeybees do double duty. They are programmed to gather pollen and carry it back to the hive as food for the

colony. However, even more,important as far as humans are concerned, they are also responsible for the pollination of more than 80 percent of green growing things. As bees buzz from blossom to blossom, microscopic pollen particles coat their stubby little bodies so densely that they sometimes look like little yellow fuzz balls. When they arrive at the next flower, a portion of the live golden dust is transferred to that blossom and pollination is accomplished. It is important to recognise that a one teaspoon dose of pollen takes one bee working eight hours a day for one month to gather. Each bee pollen pellet contains over two million flower pollen grains, and one teaspoonful contains over 2.5 billion grains of flower pollen. One bee colony gives one to seven kilograms of pollen a year. Each day, the amount of pollen collected from one colony amounts to 50–250 grams. There are special devices, or pollen traps, that are used to collect pollen baskets as field bees return to their hives (figure below). The bees must force their way through the traps to get into the hive, and they lose part of the pollen basket, sending them back out to collect more pollen.

Pollen Composition

The exact chemical composition depends on the plants the worker bees gather the pollen from and can vary from hour to hour, day to day, week to week, colony to colony, even in the same apiary, with no two samples of bee pollen identical. Accordingly, chemical and nutritional analyses of bee pollen apply only to the specific samples being tested, and cannot be

extrapolated to samples gathered in other places or other times. In its composition, there are about 250 substances, including amino acids, lipids, vitamins, macro- and **micronutrients**, and flavonoids. Although there is no specific chemical composition, the average composition is said to be 40–60% simple sugars (fructose and glucose), 20–60% proteins, 3% minerals (including calcium, phosphorus, magnesium, sodium, potassium, iron, copper, zinc, manganese, silicon and selenium) and vitamins (including water-soluble B_1, B_2, B_6 and C as well as fat-soluble vitamins A, E and D), 1–32% fatty acids, and 5% diverse other components (see table and figure below). A study of bee pollen samples showed that they might contain 188 kinds of fungi and 29 kinds of bacteria (Black, 2004). Despite this microbial diversity, stored pollen is a preservation environment similar to honey, and contains consistently low microbial biomass.

Component	Bee Pollen	Bee Bread	RDI for 15 g[a]
Proteins	7-40%	14-37%	5-22%
Carbohydrates	24-60%	24-34%	1-4.6%
Lactic acid	0.56%	3.2%	-
Lipids	1-18%	6-13%	0.1-4%
Cellulose	3.7%	2.7%	-
Flavonoids	0.2-2.5%	nd	0.03%
Vitamin	0.02-0.7%	nd	2-70%
Nucleic acid	0.6-4.8%	nd	-
pH	3.8-6.3	4.3	-

Table 12. Pollen and bee bread and human nutritional requirements (Kieliszek et al., 2017)

Campos*et al.*, 2010; Required Daily Intake requirements are according to Reports of the Scientific Committee for Food, 2010. Average RDI values have been assumed

Bee Pollen Benefits

Medicinally it is antifungal, antiviral, antibiotic, antiallergic, antimicrobial, anti-inflammatory, hepatoprotective, anticancer, immuno-stimulating, local anaesthetic and modulates the burn wound healing process (see figure below).

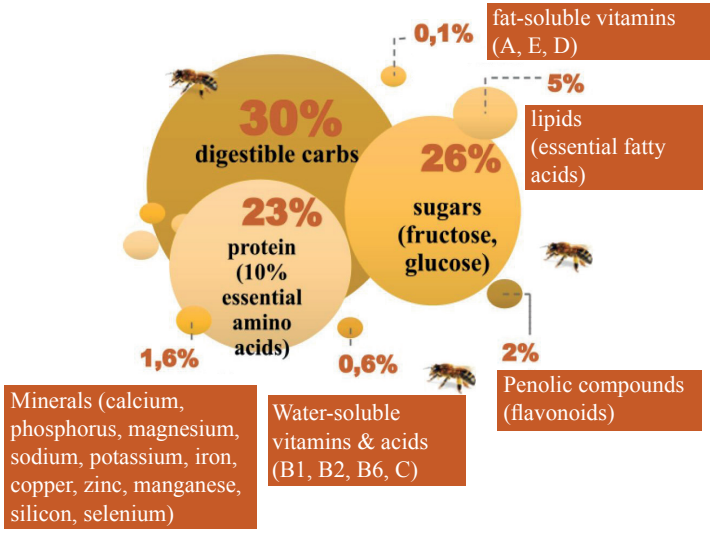

Fig. 25. The chemical composition of bee pollen (https://draxe.com/bee-pollen/)

• Reduces Inflammation

The anti-inflammatory activity of bee pollen has been compared to drugs, such as naproxen, analgin, phenylbutazone and indomethacin. Researchers suggest that it can be used in acute and chronic inflammatory conditions, initial degenerative conditions, and **liver disease**or toxicity. A study by Küpeli *et al.* (2010) found that honeybee pollen displayed significant anti-inflammatory activities when given to mice with **acetaminophen**-induced liver necrosis. Another study conducted by Maruyama *et al.* in 2010 investigated the anti-inflammatory effect of bee pollen bulk, its water extract and its ethanol extract by a method of **carrageenan**-induced paw edema in rats. The results indicate the bulk mildly suppressed the paw edema while the water extract showed almost no inhibitory activity. The ethanol extract showed potent anti-inflammatory activity, and researchers suggest that it can be used as a dietary supplement and as a functional food.

• Acts as an Antioxidant

Recent studies have revealed that enzymatic hydrolysates from bee pollen are beneficial for patients undergoing various diseases, such as cancer, cardiovascular diseases, diabetes, and hypertension. The antioxidant properties were measured, and researchers found that it has remarkable antioxidant activity. They witnessed high scavenging activities against active oxidative stress. Researchers even suggested that the

inhibitory activities of bee pollen were like those found in **fermented foods**, such as natto, miso, cheese and vinegar (Nagai *et al.*, 2005).

• Protects against Liver Toxicity

Yıldız *et al* (2013) found that the chestnut bee pollen protects hepatocytes from the oxidative stress and promotes the healing of liver damage caused by toxicity. Rats with carbon tetrachloride-induced liver damage were separated into two groups – one group took two different concentrations of chestnut bee pollen orally (200–400 milligrams per kilogram a day), and one group was given silibinin, a medication that contains flavonoids. The researchers detected that both treatments reversed the liver damage, but silibinin caused significant weight loss and death due to severe diarrhoea when given to rats. These findings suggest that bee pollen is a safe alternative to the silibinin in the treatment of liver injuries and can be part of a **liver cleanse.**

Boosts the Immune System

Bee pollen has antimicrobial and antiviral properties. A study evaluated the biological actives of eight commercial bee pollen purchased from the market. All of the samples exhibited antimicrobial activity. *Staphylococcus aureus* was the most sensitive to bee pollen, and ***Candida****glabrata* was the most resistant (Pascoal *et al.*, 2014).

• Natural Allergy Fighter

Bee pollen may also be a **natural allergy fighter**. A study conducted in Japan (Ishikawa *et al.*, 2008) investigated the effect of bee pollen on mast cell activation, which plays a central role in various allergic diseases. The researchers performed in vivo and in vitro experiments and found that bee pollen does have anti-allergic action because of its ability to inhibit the activation of mast cells, which plays an essential role in the early and late phases of allergic reactions.

- **Serves as a Dietary Supplement.** Animal studies suggest that bee pollen can be used as a valuable dietary supplement. Studies have proved that mice and rats fed with pollen showed a higher vitamin C and magnesium content in the thymus, heart muscle and skeletal muscles. They also had a higher hemoglobin content and more significant number of red blood cells after pollen consumption. Bee pollen has lengthened the life span of experimental animals. The effects of bee pollen on 40 New Zealand white rabbits were evaluated. The rabbits were equally divided among four groups that received the same commercial diet. Each group was given a water solution containing no bee pollen or 100, 200 or 300 milligrams of bee pollen per kilogram of body weight. The female rabbits were mated with non-treated male rabbits from October to February and May to September. For each season, 80 weaned rabbits

originated from the females of the control group, and they were divided into the same four groups to begin treatment. Bee pollen treatment for the female rabbits at 200 milligrams significantly increased body weight, conception rate, milk yield and litter size. It also improved biochemical profiles of blood. The same dose of bee pollen also significantly increased the growth of baby rabbits and their survival rate until weaning. Similar bee pollen benefits were displayed in a 1994 study that involved pregnant rats and fetal growth (Attia *et al.*, 2011). These animal studies suggest that bee pollen has a high nutritional value and works as a supplement for animals with nutritional deficiencies. Researchers suggest that it can be helpful when given to children who have a lack of appetite or experience a developmental delay. It may also help malnourished children and adults, especially before and after surgery, when recovering from an addiction to alcohol, or when they are under physical or mental stress.

- **Relieves Menopausal Symptoms**

A 2015 study conducted in Germany found that both honey and bee pollen honey improved menopausal complaints in breast cancer patients on anti hormonal treatment. Over two-thirds of the patients who completed the study reported an improvement in their symptoms. Researchers suggest

that bee pollen and honey may be offered to women who have failed to respond to other alternatives to cope with postmenopausal symptoms. They also note that the flavonoids found in honey and pollen have been found to prevent breast cancer, supporting the use of these products in women with **menopause symptoms** and problems with or without a history of breast cancer (Münstedt *et al.*, 2015).

• Helps Relieve Stress

Because of bee pollen's nutritional and tonic properties, it improves blood supply to nervous tissue, boosting mental capacity and strengthening the nervous system that may be weakened by stress. That makes it one of the most effective natural **stress relievers**. It may be particularly useful for people with a lack of energy, especially the elderly. Even small doses of bee pollen over an extended period can improve mood and physical endurance, thereby strengthening one's desire to live. It also serves as a local analgesic, giving it the ability to relieve pain that can be brought on by stress or injury (Komosinska-Vassev *et al.*,2015).

• Promotes Healing

Bee pollen can be used as a topical ointment to speed up the healing process, especially useful as a **home remedy for burn relief**. The pollen includes kaempferol, which inhibits the activity of enzymes after a burn and decreases inflammatory reactions and swelling. Pollen helps improve blood circulation

in the vessels, and it moistens the skin. The anti-inflammatory and analgesic action of flavonoids in bee pollen helps relieve pain and prevent platelet aggregation. Pollen also helps prevent infection because of its antimicrobial activity, allowing a wound or burn to heal quickly (Komosinska-Vassev, 2015). bee pollen is a great source of many vitamins and minerals, it can also help keep skin looking younger and glowing. It stimulates blood supply to all skin cells, helps detoxify the body, reduces the appearance of wrinkles and speeds up the healing process.

Routes of Administration and Dosing

In adults, 20–40 g is applied therapeutically every day. If a teaspoon is 7,5 g of pollen, it can be concluded that one dose is 3–5 teaspoons of this product for adults and 1-2 teaspoons for children. Pollen is usually taken 3 times a day before eating. The time of treatment is 1–3 months, but it can be repeated 2–4 times a year. The most appropriate period for treatment is between winter and spring and between summer and autumn. Generally, a smaller dose of pollen is used in combination therapy, alongside other medications and in chronic diseases (Bogdanov, 2014).

Bee bread, as a product of a stronger action than pollen, is usually administered in smaller amounts or for a short period. Romanian researchers, in the therapy of chronic hepatitis, gained the same results for bee bread used in the amount of 30 g daily during a month and for pollen in the same dose administered for 3 months.

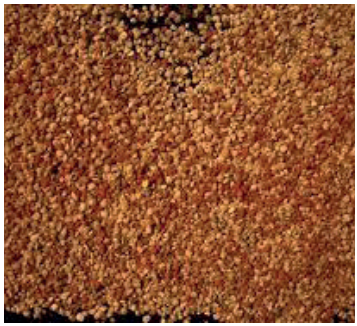

Photo 21. Harvesting pollen

To increase the digestibility of the organism, pollen grains are shredded by grinding or are subjected to warm water. In the water environment, pollen grains become swollen and, after 2-3 hours, crack and, consequently, release their values. Milk, fruit, and vegetable juices are also used for this purpose. (Ground) pollen may be mixed with many products in the ratio from 1:1 to 1:4 with the use of honey, butter, cottage cheese, yogurt, jams, glucose, and others. Mixed pollen is taken in the amount of 1 teaspoon 3 times a day. In many diseases, however, enzymatic pollen is recommended for use (see figure below). To sum up, it should be emphasized that unshredded pollen, accurately chewed before swallowing, is used by the organism only in about 10–15%. After mechanical shredding or natural release, the accessibility of natural pollen increases to 60–80% (Bogdanov, 2014).

Bee Pollen Side Effects

Get emergency medical help if somebody has any of these signs of an allergic reaction: hives, itching; feeling light-headed; difficult breathing; swelling of somebody's face, lips, tongue, or throat. Although not all side effects are known, bee pollen is thought to be possibly safe when taken for up to 30 days. Long-term use of bee pollen may cause serious side effects. Stop using bee pollen and call somebody healthcare provider at once if somebody has:

- ✓ Skin rash, bruising, severe tingling, numbness, pain, muscle weakness.
- ✓ Trouble breathing.
- ✓ Upper stomach pain, loss of appetite; or
- ✓ Swelling, rapid weight gain.

Common side effects may include:

- ✓ Numbness, tingling; or
- ✓ Upset stomach.

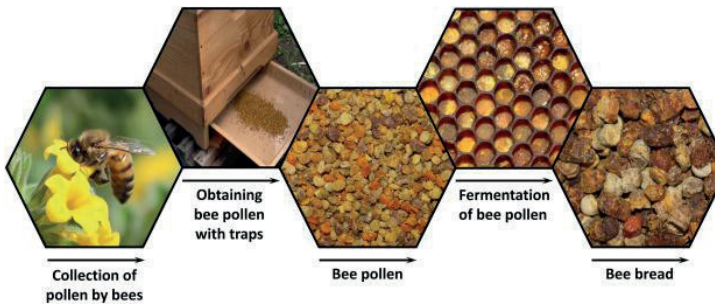

Fig. 26. Bee pollen and proceeding

Bee Bread

Assoc. Prof. Dr. Anželika Dautartė
VMU - Dept. of Environment and Ecology,
Faculty of Forest Sciences and Ecology - Lithuania

What is bee bread, and how is it made?

An increasing number of people appreciate the therapeutic effect not only of honey but also of other products with wide application in apitherapy. Bee bread (ambrosia) is a unique product, which is very important not only for humans but also for the bees. It is not always easy to get it, and the price is several times higher than the price of honey. The bee bread mainly includes pollen, honey, and secretions of bees' salivary glands (Fig. 9) (Barajas *et al.*, 2012; Vasquez & Olofsson, 2009). Bee bread is an "alchemical" bee creation made with around 25% honey or nectar, 70% pollen, and bee saliva, which, alongside the honey/nectar, inoculates the pollen with a broad range of natural probiotic bacteria and yeasts, all necessary to kick start the essential act of fermentation and predigesting. The in-hive bees tightly pack the pollen into the comb-cells and intermix it with the other ingredients. After a few weeks, a significant transformation has taken place. The bee bread has been made.

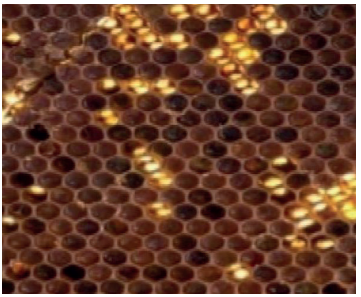

Photo 22. Bee bread in the comb (photo by Josh Pollen)

Photo 23. Pollen of various colours stored in the cells of a honeycomb near the brood. Some larvae can be seen, most of the brood cells are already capped (photo by Waugsberg)

The bees do not consume their pollen fresh. Instead, they take it into the hive and pack the granules into empty comb cells, mixing them with nectar and digestive fluids and sealing the cell with a drop of honey. Once processed in this way, the pollen remains stable indefinitely. Beekeepers call this form of pollen ‘perga’ or ‘bee bread’. Fresh pollen is high in moisture and protein and, especially when brought into the hive which stays around an internal temperature of 37˚C – becomes an ideal environment for mould growth.

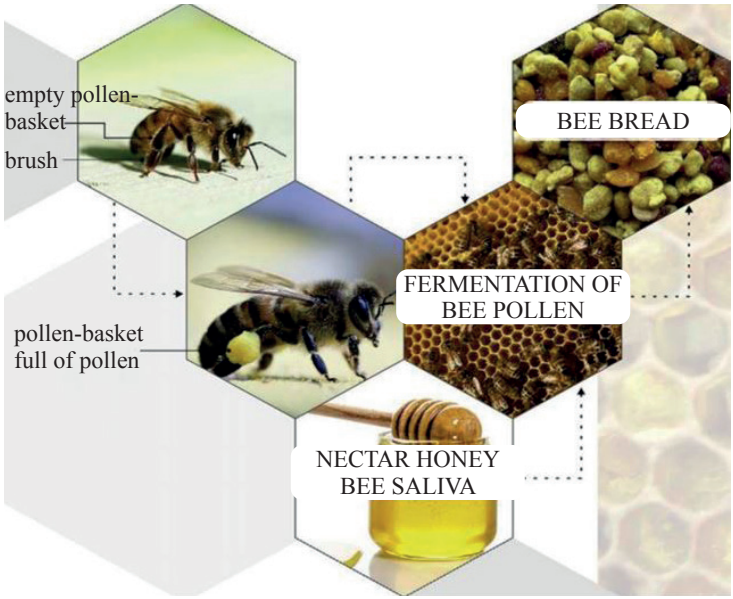

Fig. 27. The process of making a bee bread (Kieliszek et al., 2017)

Photo 24. Pollen packed in the comb cells (photo by Kyle Vialli)

The bees' digestive fluids, however, are rich with lactic acid bacteria (LAB) (Vásquez and Olofsson 2009), which come to dominate the pollen substrate when it is packed together and sealed from the air with honey. The bacteria metabolise sugars in the pollen, producing lactic acid and

lowering the pH from 4.8 to around 4.1 (Mattila *et al.* 2012) well below the generally recognised threshold for pathogenic microbial growth of 4.6. These LAB come predominantly from the bees themselves, rather than, for example, the plants from which they forage (Gilliam 1979a; Gilliam 1979b), and the difference in microbial ecology of fresh pollen vs stored is great (Gilliam *et al.* 1989). Furthermore, many of the genera which come to dominate fermented pollen are also some of those most common in fermented food products made by humans: *Oenococcus*, *Paralactobacillus*, and particularly *Bifidobacterium*, a known probiotic genus whose activity in bee hives has also been correlated with lower counts of pathogenic microbes (Mattila *et al.* 2012). Beneficial yeasts and fungi have also been documented in bee bread (Gilliam 1979b; Gilliam *et al.* 1989). Many of these beneficial fungi are susceptible to fungicides in the environment (Yoder *et al.* 2013), often applied to plant crops. Greater microbial diversity of beneficial microbes in bee colonies has also been correlated with the greater genetic diversity of the bees themselves, and this symbiosis between bees and their microbes, like in humans, is becoming increasingly studied as a likely fundamental part of overall hive health (Mattila *et al.* 2012). In addition to the preservation (Anderson *et al.* 2014), the fermentation process of the pollen also renders its nutrients more available (Mattila *et al.* 2012). Some proteins are broken down into amino acids, starches are metabolized into simple sugars, and vitamins become more bioavailable (Degrandi-

Hoffman, Eckholm, and Huang 2013; Herbert and Shimanuki 1978). In this sense, bee bread is even more health-giving than the more commonly available fresh bee pollen. The sensory transformation of the bee pollen into bee bread might be most remarkable. The floral and herbal notes of individual granules become enhanced; the powdery, sandy texture becomes firmer and moisture; the acidity from the lactic acid brightens the flavour and tempers possible bitterness; and the fermentation also produces secondary aromas that generate new flavors of fruit – some, for example, gain the distinct taste of mango. The particularities of the fresh pollen, depending on the season and its plant sources, become enhanced, and new qualities that were not present before are revealed. The different coloured balls of pollen packed in the comb cells at the beginning of fermentation (see figure below). As the fermentation process develops, the colours infuse, and the pollen walls are broken down.

So how does bee pollen compare to bee bread? Probably the most important change concerns the protein. Not only has the protein quality improved – i.e. protein bioavailability is significantly enhanced, but many proteins have also been predigested into their constituent amino acids making absorption significantly easier. From the perspective of protein quality (digestibility), bee pollen can't touch bee bread. Inside of the bee bread, many vitamins have also increased in value and Vitamin K is present for the first time. Both antioxidant concentrations and enzyme levels are also significantly elevated. What is more,

much of the potent nutrient reserves in "hibernation" within the pollen are now plentifully available; this is especially true of minerals such as zinc, magnesium, and silica, which are often bound tightly within the cellulose portion of the pollen. Bee bread is a potent, energy-rich food. Even the lactic acid created by the probiotic bacteria is converted into glucose within our bodies. A further and equally important advantage of bee bread is that it vastly prolongs the lifespan of the pollen. You see fresh pollen has an exceedingly short lifespan, and pollen will die very quickly if not properly stored (e.g. freezing). Moreover, yet, the bees have worked out how to prolong the pollen's lifespan for well over a year through the manufacturing of bee bread. This is what the bee bread looks like when it is harvested, notice it has taken the shape of the comb-cells. Bee bread is different from fresh pollen, and it contains more sugars and much less starch. According to Roulston and Cane (2000), the content of starch in pollen is in the range of 0-22%. Most kinds of pollen contain less than 5% of starch, and pollen derived from sunflowers contains only 0.4% starch. Bee bread is rich in B vitamins, as well as vitamin K, which is not present in the fresh pollen (Gilliam, 1979a). The content of carotenoids in bee pollen derived from Latvia ranged from 6.7 to 9.3 mg/100 g. The content of lactic acid, which is a preservative agent, in bee bread is higher than 3%. The content of lactic acid in bee bread coming from birch pollen is six-fold higher than in the pollen. Carbohydrates constitute between 24 and 34% (Barene *et al.*, 2015). Bee bread is more biologically active and easily digestible due to the high

content of easily digestible sugars, fat, mineral components, and a higher proportion of free amino acids when compared to pollen (Nagai *et al.*, 2004; Trzybinski, 2005). Bee bread may be a beneficial food product for people working mentally (Nagai *et al.*, 2004). Any negative changes in nutritional habits between the bee bread and pollen were demonstrated. Currently, the scientific research conducted proved that the bee products played a huge role in the detoxification process stimulation. Under their influence, harmful substances accumulated in an organism are converted to water-soluble compounds that can be easily removed from an organism (Estevinho *et al.*, 2008; Almeida-Muradian *et al.*, 2005).

- **Strengthens the Immune System**

Bee bread strengthens the immune system of an organism and also supports the treatment with pharmaceuticals. It also improves concentration and memory. It can be used during an increased mental effort. Also, it is also used in apitherapy, that is, treatment using the products of bee origin. Bee bread demonstrates an effect regulating the digestive system functioning. Due to its antimicrobial properties, bee bread is recommended especially during the periods of reduced immunity, for example, in autumn-winter season.

- **Reduces Allergic Reactions**

Moreover, it reduces allergic reactions. Therefore, bee bread should be used before the period of pollination. It also

regulates the cholesterol level in the blood and reduces total lipid content, which proves that bee bread has anti- atherosclerotic activity and also is beneficial for the heart. Also, it demonstrates an anti-ageing and anti-anaemic activity, among other things, because of the presence of antioxidants in it, and regenerates all cells of the body. Bee bread is widely used in the purification of the liver, acts protectively and detoxifies.

- **Treats and Prevents of all Types of Extravasations**

Because bee bread contains vitamin K (Gilliam, 1979a, b), it is beneficial in the treatment and prevention of all types of extravasations, as well as problems arising from the poor condition of the blood vessels (Nagai *et al.*, 2004). The therapy using the products containing vitamin K is often recommended after laser surgery-it effectively and quickly reduces bruises formed on the skin. In China, bee pollen from *Brassica campestris* L. is commonly used as a food additive to enhance the organism's immunity against cancer diseases (Omar, Azhar, Fadzilah, & Kamal, 2016). Wang *et al.*'s (2013) study demonstrated that the components of pollen, as exemplified by polysaccharides, exhibit significant antiproliferative activity in colon cancer cell lines.

- **Chemotherapeutic Agents**

Bee pollen can be used to supplement chemotherapeutic agents due to its antiproliferative activity and its ability to

enhance the chemo- an effect even at low concentrations. The molecular mechanism of how bee pollen has an antiproliferative effect will be a very interesting area to explore in future research. In Komosinska-Vassev and *et al.*'s (2015) recent review, the addition of bee pollen can improve early prostate cancer including chemotherapy. Also, as a supplement, bee pollen may be combined with chemotherapy to treat cancer side effects. Ugar *et al.*'s (2016) studies showed that bee pollen affects apoptosis and caspase-3 activity in HL-60 cells. This statement indicates that apiculture products can have beneficial effects in the treatment of cancer.

• Control Some Reproductive Processes

Bee pollen can potentially be used to control some reproductive processes. The data obtained may be not only physiological but also practical. Bee pollen affects secretory activity (release of growth factor IGF-I and steroid hormones progesterone and estradiol) (Kolesarova *et al.*, 2013). There are also reports of bee pollen's ability to induce apoptosis and stimulation of tumor necrosis factor a (TNF-a) secretion (Rzepecka-Stojko *et al.*, 2012). Also, due to the activity of substances characterized by bee pollen's antioxidant properties, there may be antineoplastic effects. Such substances affect the inhibition of the formation and removal of reactive oxygen species (ROS) (Denisow, B., & Denisow-Pietrzyk, 2016). Each bee product is pharmacologically active and may, therefore, be the source of many active substances. Of particular importance

are the new products derived from bee products with specified pharmacokinetics and pharmacodynamics, which may be the basis for many new forms of drugs or dietary supplements. In the last few years, natural products like bee bread or pollen can be used as an alternative to antibiotics, as well as to enhance the immune system of humans and animals (Farag & El-Rayes, 2016). It was demonstrated that bee pollen acts as an immunomodulator in that it stimulates a humoral immune response and changes the delayed-type hypersensitivity.

Check Yourself

1. Pollen is only produced by flowering plants.

a) true
b) yes but only trees
c) false
d) pollen is not produced by plants but by bees

2. All types of pollen are found in the air.

a) there is no pollen in the air
b) true
c) false
d) all answers are incorrect

3. Is the pollen from certain plants more allergenic than others?

a) yes
b) no
c) pollen is not allergic
d) pollen coukd be allergic only in winter season

4. The size and shape of pollen grains make them allergenic.

a) yes
b) no
c) only shape
d) only size

5. When does pollen occur in the air?

a) spring
b) summer
c) fall
d) all of the above

6. Are there specific times of the day when pollen is most abundant in the air?

a) early morning
b) mid-day
c) late at night
d) none of the above

7. I am allergic to ragweed pollen. How can I avoid exposure?

a) Stay indoors as much as possible during the ragweed season
b) Keep your windows closed at home and use an air conditioner if possible
c) Use the medication prescribed by your allergist
d) All of the above

8. Are pollen counts the highest in the spring?

a) yes
b) no
c) maybe
d) there is no pollen in the air in the spring

9. I am allergic to several types of tree pollen and I realize that my symptoms start earlier some years and later in others. Does the weather affect when the pollen season starts?

a) yes
b) no
c) maybe
d) all answers are correct

10. Pollen is made of what?

a) particles of dust
b) bee excrement
c) reproductive cells
d) vegetative cells

Answers: 1c, 2c, 3a, 4b, 5d, 6b, 7d, 8c, 9a, 10c

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Apilarnil

Prof. Dr. Kemal ÇELİK
Çanakkale Onsekiz Mart University

Apilarnil What Is It?

Apilarnil is another natural product of honeybees discovered by the famous beekeeper in Romania (Mr. Nicolae Iliesiu). It mostly has a homogenous and milky consistency of yellowish gray color with a sour taste (Bărnuţiu *et al*. 2013).

The name "Apilarnil" represents these words:

- ✓ API = the bee's name in Latin APIS
- ✓ LAR = larvae
- ✓ NIL = initial name of Nicolae ILiesu.

Apilarnil '' is another very important natural product obtained from the bee colony. The product contains drone larvae comb cells 7 or 8 days old as well as small quantities of honey, propolis, bee bread and royal jelly. Nevertheless, the main component is drone larvae, therefore the Apilarnil can be considered as the male version of royal jelly (Iliesiu, 1991). Production of Apilarnil in hives is obtained via application of frames seeded with drone (male) eggs by the queen. The process of achieving this product includes the continuous

increase of drones in certain larval stages and afterwards it is followed by the harvest. The optimal production period of apilarnil starts from fruit trees blossoming (April – May), and lasts until the end of July or beginning of August.

Apilarnil Can be produced in following forms:

- ✓ Raw (unfiltered and non-homogenized);
- ✓ Processed (filtered and homogenized);
- ✓ Lyophilized (Rodica Pana *et al*. ,2016)

Chemical Composition

From a chemical point of view, the apilarnil contains water (69 – 76 %), ash (under 1%), two major sugars (fructose and glucose) as well as minority sugars (turanose, maltose and isomaltose) and protein content (Bărnuţiu *et al*. 2013). The main amino acids found in apilarnil: leucine, isoleucine, lysine, histidine, serine, arginine, glutamic acid, tyrosine, fenilalanine, valine alalnine and methionine (Margaoan *et al*., 2017). According to Hryniewicka *et al*., (2016), homogenate of drone bee larvae contains coenzyme Q-10. Moreover, apilarnil is rich in sex hormones such as testosterone, estradiol, progesterone, and prolactin (Erdem and A. Özkök 2017)

Minerals

- Calcium
- Magnesium
- Phosphorus
- Iron
- Manganese
- Copper
- Zinc
- Sodium
- Potassium

Vitamins

- A vitamin
- Beta-carotene
- Xanthophyll
- B1 Vitamin
- B2 Vitamin
- B6 Vitamin
- PP Vitamin
- Choline

(Strant *et al*., 2015)

Curative And Therapeutic Properties

- ✓ antiviral as in royal jelly
- ✓ stimulates anabolism.
- ✓ increases the power of the immune system.
- ✓ bio-stimulant.
- ✓ improves memory.
- ✓ increases the intellectual performance of children in elementary schools.
- ✓ improves menstrual cycle for women.
- ✓ increases the appetite.
- ✓ increases the overall resistance to diseases.
- ✓ increases the body's energy, vitality, and regenerative power.
- ✓ psychostimulant
- ✓ recommended for the treatment of metabolic diseases (diabetes, fatigue, obesity, gout asthenia, chronic fatigue syndrome; diseases of the liver, stomach, and digestive tract; infections, nervous system disorders; insomnia; premenstrual syndrome etc. (Rodica Pana *et al*. ,2016)

Many other studies proved that apilarnil has many important properties such as an immune system enhancer, anabolic stimulator, body´s energy, vitality, antiviral and regenerative power (Iliescu, 1993; Stangaciu, 1999). Furthermore, as its origin is predominantly from male structure, it has rich content of androgenic hormones and therefore stimulates spermatogenesis (Constantin, 1989; Iliescu, 1993) in males. It is supposed that apilarnil can possess both anabolic and androgenic effects and could be a natural alternative to chemicals and drugs which support sexual development (Altan *et al*. 2013). According to authors Erdem and Özkök (2017) product Apilarnil "can still be used as testosterone booster food supply for sportsmenor men who are mildly troubled with andropause, and it is also advantageous since it has no known side effects in the literature to date". In addition, the product is useful in improving the aspects related to sustaining a successful job interview: increasing self-confidence, verbal fluency, and social networking ability (Gavrila-Ardelean and Gavrila-Ardelean, 2017).

Apilarnil Dosage

Consumers should be very careful with first time usage due to the possibility of allergy existence, and it should be consumed in small quantities at first. Consumers without allergy can follow general instructions:

- adult daily dose : 300 mg
  (600-900 mg, if necessary).
- children daily dosage : 30-50% of adult dose.

In case of mouth/throat or gastrointestinal form of consumption, it is recommended to use apilarnil in lyophilized form and dilute in saliva 2 – 5 minutes before swallowing. In addition, it is recommended to stop usingapilarnil after 1 or 2 months of treatment. In terms of products containing apilarnil e.g. solution, preparation, it is recommended to keep products in the refrigerator since apilarnil is unstable at high temperatures and its lifetime decreases rapidly (http://apilarnil.com/referenses.html, 2018).

Collection, Processing and Storage of Apilarnil

Apilarnil is obtained from bee larvae, that are chopped and lyophilized. (lyophilize = the conversion of water from a frozen state to a gaseous state without going through a liquid state. The freeze-dry process removes moisture from the cells of specimens while the specimens remain frozen. (according to microbiologics.com). Available most commonly in a powder form or mixed into honey in a paste, it needs to be kept frozen to ensure the substance remains active.

Harvesting Apilarnil

How can we harvest APILARNIL?

- The nest must be well coordinated.
  - the queen must have enough space to lay eggs.
  - use specific frames for drones which comes after the last frame with eggs and brood bee Harvesting apilarnil

- pressing apilarnil with comb - centrifugation
- extracting piece by piece Filtering Apilarnil is mandatory!

- APILARNIL must be harvested in maximum hygiene conditions and frozen every 30 minutes during the harvest.
- Frames with Apilarnil can sit outside the hive maxim 30 minutes.
- The ustensils what we used in apilarnil production should be disinfected (boiled) after every extraction. Transportation must be done in conditions of freezer temperature: minim –10 degrees Celsius


Photo 25. Harvesting apilarnil

Processing of Apilarnil

- Apilarnil freshly harvested from opened or unsealed cells from the bee hives. All the cells were filled with clean water and then the larvae were shaken

out (Schmidt and Buchmann, 1992). Since larvae defecated just before pupation; larvae were washed in clean water before further processing. Pupae had clean, empty intestines. Apilarnil was packed and the samples transferred to the laboratory. The samples were triturated, homogenized, filtrated, and finally lyophilized using CHRIST Alpha 1-4 LD plus (Germany). The lyophilized samples were stored at -20 ºC until analyzed.

- Moisture, total lipids, crude protein contents of the samples were determined using the AOAC method (Helrich, 1990). Ash contents were defined by placing the sample inside the incineration oven at 550 ºC for 6h, until a white powder was obtained. The crucible was weighted at the beginning and at the end. The difference was expressed in the percentage alteration of the ash content from the beginning to the end. The Kjeldahl method with distillation parameter optimization (Digester K-424, Distiller KjelFlex K-360 and titrator Schott Titro Line) was applied to evaluate the total protein contents of the sample. Fatty acid methyl esters (FAMEs) were prepared according to ISO 12966-4 (Anonymous, 2015), Supelco 37 component FAME mix was used in the internal standard.

Extracted Drone Larvae

Drone Larvae in Honeycomb

Photo 26. Drone larvaes

In conclusion, apilarnil which stimulates growth and sexual development thanks to its androgenic hormones is a member of natural bee-products. Also, it is suggested as a natural anabolism stimulator in males by the reason of its impact on the increment of the muscular bodyweight. Besides in-vitro examination, the further in-vivo studies are also needed to evaluate the potency of the androgenic and anabolic effect of apilarnil.

Check Yourself

1. The main constituent of Apilarnil is:

a) honey
b) proplis
c) drone larvae comb cells
d) royal jelly

2. Apilarnil can be considered as

a) the male version of royal jelly
b) the female version of royal jelly
c) diluted royal jelly.
d) dried royal jelly

3. The optimal production period of apilarnil starts:

a) from October, and lasts until the beginning of December.
b) from April – May, and lasts until the end of July or beginning of August
c) production does not depend on season.
d) from January, and lasts until the end of March.

4. The water content in fresh raw apilarnil is:

a) 30 - 40%
b) 90 - 95%
c) 20-40%
d) 69 – 76 %

5. The main sugars in apilarnil are:

a) maltose and fructose
b) fructose and glucose
c) lactose and glucose
d) galactose and fructose

6. Apilarnil is rich in hormones such as:

a) testosterone
b) estradiol
c) progesterone
d) all answers are correct.

7. Apilarnil possess:

a) anabolic effects
b) androgenic effects
c) both anabolic effects and androgenic effects
d) neither anabolic effects nor androgenic effects

8. Apilarnil has many important properties such as:

a) regenerative power
b) an immune system enhancer
c) antiviral activity
d) all answers are correct

9. The recommended adult daily dose of apilarnil is:

a) 30 mg
b) 300 mg
c) 3 g
d) 30 g

10. It is recommended to stop using apilarnil :

a) 1 or 2 months of treatment
b) 1 or 2 weeks of treatment
c) 6 months of treatment
d) 1 year of treatment

Answers: 1c, 2a, 3b, 4d, 5b, 6d, 7c, 8a, 9b, 10a

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BeesWax

Assoc. Prof. Dr. Barbara Król Dr. Maja Słupczyńska
Uniwersytet Przyrodniczy we Wrocławiu - Poland

Bee Wax – What's It and How Is It Made

The word wax describes a large variety of substances of plant and animal origin, as well as man-made products which are mostly petroleum derivatives. However, natural waxes are not single substances, but a mixture of various long-chain fatty acids and a variety of other constituents, depending on their origin. Each wax therefore has unique physical and chemical characteristics which are exploited in a multitude of applications. Wax from the honeybee has an extremely wide spectrum of useful applications and occupies a very special position among waxes. Young bees in the hive, after feeding the young brood with royal jelly, take part in the construction of the hive. Engorged with honey and resting suspended for 24 hours together with many other bees in the same position, 8 wax glands on the underside of the abdomens of the young bee's secret small wax platelets. These are scraped off by the bee, chewed and masticated into pliable pieces with the addition of saliva and a variety of enzymes. Once chewed, attached to the

comb, and re-chewed several times, they finally form part of this architectural masterpiece, a comb of hexagonal cells, a 20 g structure which can support 1000 g of honey.

Wax is used to cap the ripened honey and when mixed with some propolis, also protects the brood from infections and desiccation. Together with propolis, wax is also employed for sealing cracks and covering foreign objects in the hive. The wax collected by the beekeeper is that which is used in comb construction. Frame hive beekeeping produces wax almost exclusively from the cap and top part of the honey cells. For centuries, beeswax was appreciated as the best material for making candles. Before the advent of cheap petroleum-based waxes, tallow (rendered animal fat) was used for cheap candles and for the adulteration of beeswax. Ancient jewellers and artisans knew how to form delicate objects from wax and cast them later in precious metals. Colours of ancient wall paintings and icons contain beeswax which has remained unchanged for more than 2000 years (Birshtein *et al.*, 1976).

Photo 27. Bee wax

The wrappings of Egyptian mummies contained beeswax (Benson *et al.* 1978) and beeswax has long found use in medicinal practices and in creams and lotions. Of all the primary bee products it has been, and remains, the most versatile and most widely used material. Major compounds are those forming more than 1% of the fraction. The number in brackets indicates the number of compounds making up at least 1% of the unfractionated, pure wax. The number of minor compounds, those with less than 1% of the fraction, is only an estimate. The ratio of ester values to acids, a character used by the various pharmacopeias to describe pure beeswax, is changed significantly by prolonged or excessive heating. At 100^0C for 24 hours the ratio of ester to acid is changed beyond the limits set for pure bee wax. Longer heating or higher temperatures lead to greater degradation and loss of hydrocarbons (Tulloch, 1980). These changes also influence the physical characteristics of the wax. Thus, excessive heating during rendering or further processing changes the wax structurally and alters the beneficial characteristics of many of its minor compounds, not only the aromatic and volatile compounds. Bleaching destroys at least the aromatic compounds of wax. Bleached wax no longer has the pleasant and typical aroma of wax and it can be assumed that it also lacks many of the other minor compounds. Various plants growth promoting substances, such as myricil alcohol (Weng *et al.*, N-1979), triacontanol (Devakumar *et al.*, 1986), gibberellin GA_3(Shen and Zhao, 1986) and a rape oil steroid (Jiang, 1986)

have been detected in and isolated from beeswax. Kurstjens *et al.*, (1990) describe at least 11 proteins in the freshly secreted wax scales of*A. mellifera* capensisworker bees and 13 proteins in the wax combs of*A. m. scutellate*and*A. m. capensis*. The composition of wax from Asian honeybee species is much simpler and contains fewer compounds in different proportions (Phadke *et al.*, 1969, 1971; Phadke and Nair, 1970, 1973 and Narayana, 1970). These ghedda waxes therefore cannot be used as substitutes for *Apis mellifera* wax in certain recipes. Since little is known about which compounds or mixtures cause the beneficial medicinal and dermatological effects of beeswax, no conclusions can be drawn from the composition data alone. Ghedda waxes are used locally in many of the same ways as*Apis mellifera*wax is used in other parts of the world. Meliponid waxes, which are less like honeybee wax than Ghedda wax, have been used by Amerindians for many of the same purposes, as honeybee waxes (Posey, 1978).

Description	% of fraction	Number of components in fractoin	
		Major	Minor
Hydrocarbons	14	10 (5)	66
Monoesters	35	10 (7)	10
Diesters	14	6 (5)	24
Triesters	3	5	20
Hydroxy monoesters	4	6 (1)	20
Hydroxy polyesters	8	5	20
Acid esters	1	7	20
Acid polyesters	2	5	20

Description	% of fraction	Number of components in fractoin	
		Major	Minor
Free acids	12	8 (3)	10
Free alcohols	1	5	?
Unidentified	6	7	?
TOTAL	100	74	> 210

Table 13. Composition of beeswax (after Tulloch, 1980).

Beeswax is considered safe for human consumption and has been approved as an ingredient in human food in the USA (USA, 1978). It is inert, i.e. it does not interact with the human digestive system at all and passes through the body unaltered. However, substances dissolved or encapsulated in wax are slowly released. This property is exploited in many medicinal preparations. At the same time these properties can create a problem when wax is stored near toxic chemicals and pesticides or after treatment with various drugs inside the hive. Any fat-soluble toxins can be absorbed and then released much later when the wax is consumed as food, used in cosmetics, or given to bees in the form of foundation sheets.

The Physiological Effects of Wax

Beeswax is inert and has no direct effect on humans or larger animals. However, its indirect effects can be very strong. If mixed with medicinal drugs or poisonous baits, wax preserves the active materials longer and releases them slowly. It can be used to create thin non-corrosive, non-allergenic

protective films on many surfaces from metals to fruits and human skin. Thus, it protects against external damage such as corrosion and abrasion as well as against moisture loss. It is a good electric insulator and, when saponified with borax, allows the mixture of very stable and smooth emulsions for cosmetics. Even in small concentrations it improves other formulations in the same way. A very small anti-inflammatory and antioxidant activity can be observed in beeswax due possibly to some inclusions of propolis or other minor ingredients.

The Uses of Wax Today

In the past, beeswax had a wide range of uses. Though in many cases beeswax can be replaced with cheaper, synthetic waxes, its very special characteristics, medicinal benefits, plasticity, and aroma ensure its continuing use. Many of these characteristics cannot be achieved with artificial waxes. The trend for more natural products in cosmetics may also increase its use. Presently, there is a scarcity of beeswax in industrialized countries, at least seasonally. In industrialized countries, most nationally produced wax is used by beekeepers for foundation sheets. Approximately one third of imported wax is used for cosmetics, one third for pharmaceutical preparations one fifth for candles and the rest for other, minor uses (ITC, 1978). In developing countries with traditional beekeeping methods, wax is often wasted. If it is rendered, most is subsequently exported, and only relatively small proportions are used by local manufacturers. This, however, depends very much on the

local industry. There are many possibilities for good quality products in local emerging markets and in import substitution. Adjsare (1984) listed over 150 uses of beeswax as described also in an old 1954 edition of "The Hive and the Honeybee "A few examples from the wide range of products, in which beeswax can be included, together with a few recipes for small or home-based industrial productions. There are many types of synthetic waxes available today, often with superior characteristics for special applications Apart from price and availability however, beeswax has preferred characteristics in a wide range of applications and conditions. There are very few products which consist only of beeswax or in which only beeswax can be used, but the value or characteristics of most other products are enhanced or complemented by its inclusion.

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. 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. 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 *Galleriamelonella L*.

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.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.

Wax from grains (a residue after the processing of bee waxes 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 because 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 one's 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. 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 color. 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.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 ofcolour and aroma, they can be stored inwrapping-paper 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.

How to Melt and Clean Beeswax before Using?

Many people think that melting and purifying beeswax is a headache. We'll debunk this myth in a few easy steps. Now that you have beeswax, there are some important steps you need to take before proceeding and using it further. We'll look at how to melt and purify beeswax in two different ways before using it.

Why Melt And Purify Beeswax?

Purification of beeswax through a melting and purification process may be referred to as refined beeswax. Learning how to melt and purify beeswax before using it is important for a number of reasons.

- Beeswax is usually collected by bees in an outdoor setting and is therefore susceptible to some dirt particles or even dead bees. While these are natural products of the environment, they reduce the purity and smooth texture of beeswax.
- After the honey is extracted, through proper melting and purification methods, smooth, pure beeswax can be produced for use.

Melt Beeswax

The first step in cleaning beeswax is to melt it. One way is a solar oven. However, not everyone has a solar oven

in their home or garage. However, we need to heat the wax to its melting point, which is between 144 and 147 degrees Fahrenheit.To get around this, there are two alternatives to melting beeswax that can be assembled almost anywhere.

These gifts are:

- Double boiler
- Crockpot Water Bath

Double Boiler

A double boiler method is a valuable way to melt your beeswax.

1. Gather a large pot and a small metal bowl that sits comfortably on top. Make sure you use metal bowls that you don't need to eat later or use for food preparation as beeswax is difficult to remove
2. Fill the bottom pot halfway with water
3. Bring the water to a boil, and then place the metal bowl on top
4. Now reduce the heat and bring the water to a boil
5. You can now place your beeswax in a metal bowl and watch it slowly melt. This takes at least 15 minutes

If you plan to clean the wax next or use a different molding method, we strongly recommend that you prepare the cheesecloth filter system before the wax hardens again. It is very important not to let the water touch the wax as this will destroy the natural texture. It also ensures that no direct

heat source comes into contact with your wax. This can burn you and damage your wax.

Crockpot Water Bath

Besides being useful in cooking, crock pots are also a great tool for melting beeswax.

1. Get a large clay pot and a smaller metal bowl or pitcher that fits easily in a bowl. This will hold your beeswax, so make sure it's not a dish you plan to use as food later.
2. Fill the crock pot halfway, but make sure the water doesn't spill over the top of the metal bowl/pot into the wax.
3. Bring the water to a boil and put the metal bowl in it.
4. Carefully pour the wax into a metal bowl or jar, taking care not to contaminate the wax with the water. It starts to melt slowly.

By using this melting method, you have separated clean beeswax from the dirt. If you carefully remove the bowl from the pot, you will find a plate of clean beeswax on it, and the dirty beeswax has sunk to the bottom. After either melting method, you can further purify your beeswax using a gauze filter.

Cleaning Beeswax

Once melted, this once-solid beeswax is easier to clean up in its liquid state, allowing us to work with it further than solid beeswax. Although beeswax has many antibacterial

properties, some bacteria may still be present in it until it is cleaned. Bacteria that may be present in the wax would have been destroyed by the high melting temperature.

Now is the time to clean any solid contaminants from the beeswax. There are two ways:

- Temperature method
- Gauze filter

Temperature Method

The first way to clean beeswax is to repeat the same method as melting beeswax. Melting the wax also helps separate impurities from pure wax. You may have noticed that the clean beeswax forms a sort of disc on the top of your bowl with some "dirty" residue on the bottom. Just repeat one of your smelting operations and collect clean slices from above.

Gauze Filter

Another technique for cleaning melted beeswax is a filter system. Filtration is done with cheese cloth. By using a fine screening system, you can remove every ounce of contamination in your beautiful beeswax. Not only will your wax be smoother and softer, it's also of higher quality

Simple steps are:

1. Gather your cheesecloth and a tall pitcher or large storage container into which the beeswax will flow. Ideally, this is where you want to store beeswax when you need it.

2. Place the cheesecloth tightly over the storage container and secure by tying string around the edges and around the container or with a rubber band.
3. Once the wax has melted, pour it slowly onto the cheesecloth following the steps above.
4. The clean wax drips slowly over the cheesecloth, leaving impurities on the cloth.
5. If you're cleaning a lot of beeswax, it's best to do it in small batches to prevent the wax from cooling and solidifying as it drips over the cheesecloth.

What's Next?

Cleaning beeswax has a variety of uses in the medical and cosmetic industries. It can also be used to make natural candles as well as sustainable alternatives to plastic, e.g. beeswax foil. Melting and cleaning your beeswax is certainly not as complicated as it looks! Almost anyone can do this anywhere. Choose your melting method, and then use the temperature method or cheesecloth filtration to purify the beeswax. The world is your hive!

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 adulterants. 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, paradichlorobenzene). Another potential problem for the quality of beeswax used for beekeeping is the presence of American foulbrood (*Penibacilluslarvae larvae*) spores.

1. **Today two/third of bee wax is used as:**
   a) cosmetics and pharmaceutical preparations
   b) cosmetics and food
   c) food and candle production
   d) medicals and food

2. **The most abundant compound in bee wax are:**
   a) hydrocarbons
   b) free acids
   c) monoesters
   d) diesters

3. **The ratio of ester values to acids, a character used by the various pharmacopoeias to describe pure beeswax is changed significantly by:**
   a) prolonged or excessive freezing
   b) prolonged or excessive heating
   c) floral source
   d) all answers are incorrect.

4. Bleaching of the bee wax destroys at least:

a) monoesters
b) free acids
c) hydrocarbons
d) aromatic compounds

5. Various plant growth promoting substances, such as myricil alcohol, triacontanol or a rape oil steroid have been detected in and isolated from beeswax:

a) false
b) true
c) there are no evidence.
d) plant growth promoting substances are not able to accumulate in bee wax

6. Beeswax is inert. It means that:

a) it forms with minerals unavailable forms with minerals.
b) it does not interact with the human digestive system at all and passes through the body unaltered
c) has beneficial effect on gastrointestinal tract.
d) is harmful and cannot be used as food ingredient.

7. Bee wax:

a) can be used to create thin non-corrosive, protective films on many surfaces.
b) protects against external damage such as corrosion and against moisture loss
c) is a good electric insulator
d) all answers are correct

8. A anti-inflammatory and antioxidant activity can be observed in beeswax:

a) yes
b) yes but very small
c) no
d) all answers are correct.

9. Beeswax is considered safe for human consumption and has been approved as an ingredient in human food in the USA. However, wax can be a source of some toxic or harmful substances when:

a) wax is stored near toxic chemicals and pesticides
b) after treatment with drugs inside the hive
c) wax in inert so it can provide any toxic
d) answers a and b are correct

10. The bee wax is obtained by melting of the honeycomb. The temperature during this production should not exceed:

a) 150°C
b) 200°C
c) 90°C
d) 50°Ce)

Answers: 1a, 2c, 3b, 4d, 5b, 6b, 7d, 8b, 9d, 10c

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Apitherapy - European Union Legislations

Dr. Massimo Canalicchio Dr Andrea Palomba
CONFEDERAZIONE ITALIANA AGRICOLTORI REGIONALE UMBRIA-ITALY

Bee-therapy Legislations in Europe

This report has been proceeding based on the report Legal status and regulation of CAM in Europe created as deliverables of the project CAMbrella (FP7-HEALTH-2009-3.1-3). The following EU Directives and Regulations can potentially influence national legislation regarding CAM practices, treatments and patients' rights and safety: The "Professional qualifications Directive" 2005/36/EC of 7thSeptember 2005, on the recognition of professional qualifications[1] - The Directive is the legal basis for free movement of professionals in Europe. A profession is considered regulated when access to it and the exercise of it are subject to the acquisition of a specific professional qualification. The "Patient Rights Directive" 2011/24/EU of 9thMarch 2011, on the application of patients'

1 DIRECTIVE 2005/36/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 7 September2005 on therecognition of professionalqualifications (Text with EEA relevance) (OJ L 255, 30.9.2005,p. 22) Amendedup to March 2011, (2005).

rightsin cross-border healthcare[2] - the Directive describes the patients' rights with regard to access safe and good quality treatment and to be reimbursed for it. The Directive can bear influence on CAM practices and CAM patients whether the specific treatment/practitioner is registered as conventional or non-conventional in the country of interest.

Legal status and regulation of CAM in Europe

Regulation of practice is in general mostly tied to formal education and/or training in conventional or unconventional medicine. Regulated professions who practice CAM are often divided into:

Health professionals

- ✓ **Doctors (MDs)–** in this category can be included medical doctor, medical doctor with CAM education, medical doctor with CAM licence, medical doctor with CAM authorization, physician, CAM physician, or allopathic doctor.
- ✓ **Other health professionals** - in most cases these are conventional health personnel with an educational level of 3-5 years. Nurses and midwives are the health professions most represented in this category, but also physiotherapist, chiropractor, manual therapist, osteopath, masseur and other titles in national legal

2 DIRECTIVE 2011/24/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 9 March2011, on theapplication of patients' rights in cross-borderhealthcare (OJ188, 4.4.2011, p.45),(2011).

documents, in some countries regulated as health personnel, in others as CAM practitioners can be included into this category.

- ✓ **Other CAM practitioners**– the category includes CAM practitioners with a short or no medical education or training. Classifications in the legislation include medically trained personnel (less than 3 years), non-medical personnel, paramedics, non-professional health worker, acupuncturist, herbalist, homeopath and other CAM practitioners.

General CAM legislation

Only several EU countries have a general CAM legislation, and 6 countries with CAM sections included in their general health care legislation. In addition to the general CAM legislation some countries have regulations on specific CAM treatments. Of the 15 original EU member countries, only Belgium, Germany, Portugal, from countries entered in 2004 Hungary and Slovenia and from new member countries only Bulgaria and Romania have a general CAM law. Denmark has a "law on a self-regulatory registration system for alternative practitioners" and Malta has general CAM legislation included in the general health care legislation. Austria, Denmark, Finland, France, Greece, Italy, Luxembourg, Spain, Sweden, UK and the 7 member countries who joined in 2004 (Cyprus, Czech Republic, Estonia, Latvia, Lithuania, Poland and Slovakia) have regulations on specificCAM treatments. Bulgaria and Romania have also specific CAM treatment regulations.

Consequences for European patients and citizens

With patients more willing to cross borders in their search for health care (encouraged by the recent Cross-border Health Directive[3]), it is imperative that they are aware of the widely different status of CAM legislation and regulation in culturally similar European Nations. When patients cross European borders in search of CAM treatment, they may encounter substantial differences in the professional background of apparently identicalCAM providers. They may also face a completely different reimbursement system, and if the treatment they undergo results in unwanted adverse or side effects they will be differently safeguarded depending on which state they are in. This document thoroughly documents that European patients will experience a wide variation in CAM treatment situations depending on country of residence. The patient will face the following challenges:

- ✓ A wide diversity in which treatments and providers that are available.
- ✓ A wide diversity of available treatments and providers.
- ✓ For similarly labeled treatments, a totally unpredictable level of professional competence.
- ✓ Very differing systems of authority regulation of quality of the services provided.

3 https://ec.europa.eu/health/cross_border_care/overview_en

- ✓ Unpredictable system of reimbursement for services provided.
- ✓ Limited and complex opportunities for complaints.

Every aspect of the current situation can be a threat to patient safety. In post-modernEurope where patient choice in health care is seen as a core value, this confusing Europeanmarket makes any informed treatment-seeking very challenging. It also indirectly discourages any cross-border treatment-seeking activity. For patients, health insurances and treatment providers there is an unacceptably high level of confusion.

Consequences for CAM practitioners

When CAM professions in some countries are tightly regulated while the same professional categories in other countries are totally unregulated, an establishment of collegial commonground is very challenging. Despite these challenges, there have been numerous organizations established who have attempted to coordinate international collaboration and facilitate research:

- ANME (Association of Natural Medicine in Europe),
- CAMDOC Alliance (alliance of the four major European medical CAM umbrella organizations ECH, ECPM, ICMART and IVAA),
- ECCH (European Central Council of Homeopaths),
- ECH (European Committee for Homeopathy),
- ECHAMP (European Coalition on Homeopathic and Anthroposophic Medicinal Products (E.E.I.G.)),

- ECPM (European Council of Doctors for Plurality in Medicine),
- EFCAM (European Forum for Complementary and Alternative Medicine),
- EHTPA (European Herbal and Traditional Medicine Practitioners' Association),
- EICCAM (European Information Centre for Complementary and Alternative Medicine),
- ELIANT (European Alliance for Applied Anthroposophy),
- EPHA (European Public Health Association),
- ICMART (International Council of Medical Acupuncture and Related Techniques),
- IVAA (International Federation of Anthroposophic Medical Association),
- KB (Kneipp-Bund eV).

Most of these organizations have as one of their goals to attain scientific, legal and regulatory recognition and approval of their CAM treatment modality. The current legal and regulatory landscape would most likely have appeared even more confusing without their efforts.

Authorized/Licensed Health Care Providers

For medical doctors the Directive 2005/36/EC facilitates the mutual recognition of conventional medical qualifications (basic training, additional training as general practitioners or medical specialists, if applicable). The system does, however,

not easily handle their possible additional qualifications in specific CAM therapies. The authorized/licensed health care providers with or without a local specialty can practise CAM in another state according to legislation in that specific country. However, such practice is sometimes impossible due to the heterogeneous regulation in Europe.

Obstacles can be:

- Authorizations and licences allowing the practice of CAM differ between states.
- There are differences from state o state with regard to which CAM treatments that can be provided by the authorized/licensed health care providers included in the Directive 2005/36/EC (5).
- Education and training programmes both for health professionals included in Directive 2005/36/EC (5) and for other CAM providers differ from state to state.

Consequently, a Doctor of Medicine in one state could have some training in the CAMfield included in the curriculum, while CAM training is not included in the curriculum in another state. Both curricula can, however, have been accepted according to the professionals Directive. Within the current legislation at the EU/EFTA level there is therefore room for a variety of CAM practise performed by authorized/licensed health care providers. This ranges from providers with no training in CAM practicing in a state where no CAM modalities are permitted to, at the other extreme, providers practicing in

states where there is considerable CAM training within the current curriculum, or post-graduate accredited CME training courses in several CAM modalities, or authorization/licensing of CAM specialists in the respective professions. This situation raises concerns about the predictability, quality and safety of healthcare delivery to European citizens by licensed health care providers practicing CAM.

CAM provider without an authorization /license as a healthcare provider

Directive 2005/36/EC) on the recognition of professional qualifications influences the provision of CAM treatment in Europe also for those CAM providers who are not authorized/licensed as health care personnel. A few countries have established separate authorization/licensing systems for some categories of CAM providers (for example acupuncturists and chiropractors), and these are included in the professional groups regulated by Directive 2005/36/EC. These CAM providers can seek professional recognition within the countries that regulate them. CAM providers share the basic right to work in all European states under the Directive 2004/38/EC (Rights of Union citizens to move and reside freely). However, the country-specific nature of member states' recognition of CA professions means they cannot exercise this right across all member states. They can this possibly be legally recognized in their own country, but not recognized in other EU or EFTAcountries. They are

required to follow national legislation/regulations in each state with regard to what treatment they are allowed to give and relate to the provider and insurance regulations within the state's private or public health systems. This severely hampers the free movement of providers despite the Cross-border Healthcare Directive and Social Security Regulation. Within the current legislation at the EU/EFTA level there is therefore room for a variety ofCAM practice performed by providers who are not authorized/licensed health care providers. This ranges from an extreme of being refused to practise at all, because all treatment of people with health conditions is reserved for authorized/licensed health care personnel only, to another extreme in some European countries where anyone can practice AM without any CAM education or training. Another extreme situation is being allowed to practice CAM as a fully trained provider with an authorization/license on equal terms to an authorized/licenced health care provider. This diverse situation raises, as in the case of authorized/licensed health care providers, concerns about the predictability, quality and safety of CAM health care delivery to European citizens.

1. **The EU Regulations that can potentially influence national legislation regarding CAM practices, treatments and patients' rights and safety are"**
   a) the "Professional qualifications Directive" 2005/36/EC of 7th September 2005
   b) the "Patient Rights Directive" 2011/24/EU of 9th March 2011
   c) there are no such regulations
   d) answer a and c are correct

2. **Regulated professions who practise CAM are often divided into:**
   a) doctors (MDs)
   b) other health professionals
   c) other cam practitioners
   d) all answers are correct

3. **According to directive, in category *Other health professionals* are included among others:**
   a) physiotherapist
   b) medical doctor
   c) paramedics
   d) all answers are correct

4. **According to directive, in category *Other health practitioners* are included among others:**
   a) physiotherapist
   b) chiropractor
   c) homeopath
   d) all answers are correct

5. **Of the 15 original EU member countries, only three have a general CAM law. These countries are:**
   a) Austria, Germany, Spain
   b) Belgium, Germany, Portugal
   c) Netherlands, Germany, Italy
   d) Belgium, Germany, Spain

6. **Of the 10 EU member countries that entered EU in 2004 only two have a general CAM law. These countries are:**
   a) Poland and Slovakia
   b) Poland and Czech Republic
   c) Hungary and Slovenia
   d) Slovakia and Cyprus

7. **Of the 2 EU member countries that entered EU in 2007 a general CAM law has/have:**
   a) Romania
   b) Bulgaria
   c) both Romania and Bulgaria
   d) neither Romania nor Bulgaria

8. There are ….. European countries with CAM sections included in their general health care

a) 6
b) 4
c) 15
d) 10

9. The status of CAM legislation and regulation in culturally similar European is:

a) the same in all European countires
b) widely different
c) slightly different
d) there is no CAM legislation in European countires

10. The patient in CAM treatment situations in different European countries may experience:

a) a wide diversity in which treatments and providers that are available
b) a wide diversity of available treatments and providers.
c) for similarly labelled treatments; a totally unpredictable level of professional competence
d) all answers are correcte)

Answers: 1d, 2d, 3a, 4c, 5b, 6c, 7c, 8a, 9b, 10d

ENVIRONMENTAL CONTAMINATION OF BEE PRODUCTS

Assoc.Prof. Dr. Dr. Anzelika DAUTARTE
Vytautas Magnus University-LITHUANIA

General sources of contamination of bee products

Environmental pollutants emitted by agricultural, urban, and industrial activities, as well as new infections and climate change, may have a deleterious influence on microbial, plant, and animal life. Heavy metals, ecologically persistent chemicals, and agrochemical insecticides are among the contaminants of concern for social insects (Fig. 28.). Pesticides, which include insecticides, mostly originate from agricultural activities, while heavy metals are introduced into the environment because of industrial operations, combustion, or vehicular traffic. Fine particulate matter includes both insecticides, including their residues, and heavy metals that are attached to particles measuring 10 μm or less. Fine particulate matter consists of several chemical components that have the potential to be hazardous. Social insects could orally acquire pollutants when foraging, subsequently transferring them to the brood or integrating them into nest material. Pollutants have the potential to contaminate stored food sources in bees, including honey and

bee bread. Furthermore, it is possible for contaminants to be directly deposited on the cuticles of insects and their nests by atmospheric means. Following this, these materials may once again be integrated into the structure of the nest or even enter the body of the insect, such as via the tracheal system (Feldhaar, Otti, 2020). The pollutants may have severe environmental consequences since they can deposit and migrate through soil, water, and air, accumulating in bodily tissues and causing reproductive failure, neurotoxic damage, and death (Moron et al., 2014; Williams et al., 2015). These negative consequences do not just affect the natural ecosystem. Contaminants have been linked to respiratory disorders, cancer, and other illnesses in people (Briffa et al., 2020). Real-time monitoring of environmental quality is becoming more important for recording variations and assisting in the preservation of landscape biodiversity, food security, and human health.

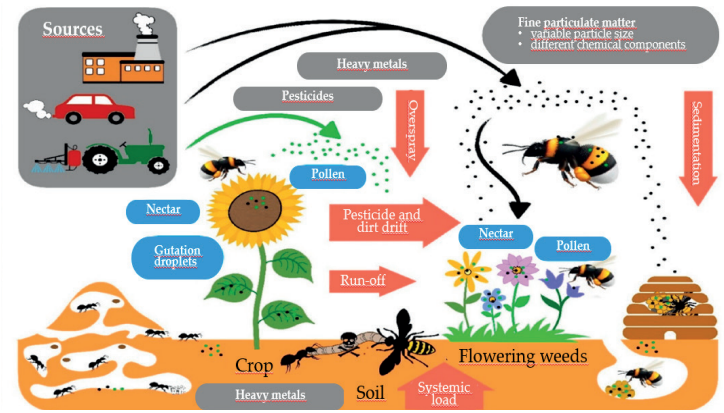

Fig.28. Sources of the environmental pollutants and exposure pathways of social insects to pollutants (source: Feldhaar, Otti, 2020).

Bee-derived products possess the inherent susceptibility to contamination originating from several sources. Contamination might arise from beekeeping practices or environmental causes (Fig. 29). Environmental pollutants include a range of substances that pose a threat to the environment. These substances include heavy metals like lead, cadmium, and mercury, radioactive isotopes, organic pollutants, pesticides (including insecticides, fungicides, herbicides, and bactericides), pathogenic bacteria, and genetically modified organisms. The technique of beekeeping gives birth to a certain quantity of contaminants. The main classifications of agents used for pest management in beekeeping include acaricides, including both lipophilic synthetic chemicals and non-toxic substances like as organic acids and components derived from essential oils. In addition, the management of bee brood diseases involves the use of antibiotics such as tetracyclines, streptomycin, sulphonamides, and chloramphenicol. In the field of beekeeping, there are supplementary substances that serve secondary roles. One such component is para-dichlorobenzene, which is used to control wax moth infestations. Additionally, chemical repellents are applied for similar purposes (Bogdanov, 2005). Contaminants have the potential to infiltrate the primary constituents of bee products, namely nectar, honeydew, pollen, and plant exudates, via several pathways such as air, water, plants, and soil. Subsequently, these contaminants may be conveyed into the bee hive through the active involvement of bees.

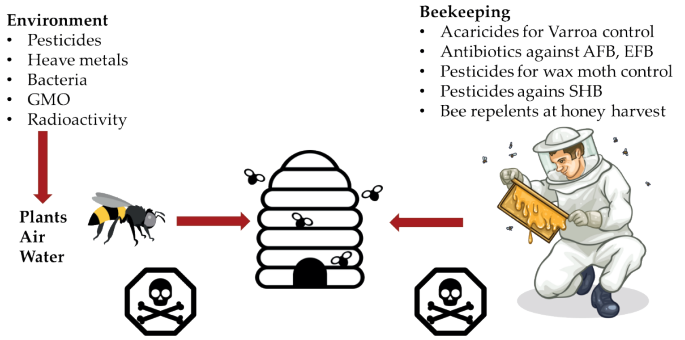

Fig. 29. The contamination sources for the bee colony. *GMO: genetically modified organisms; AFB: American foulbrood; EFB: European foulbrood, SHB: small hive beetle* (source: Bogdanov, 2005).

Heavy metals. Heavy metal pollution is a hazard in densely inhabited places, especially in industrialised areas. Vehicle exhaust, the combustion of fossil fuels, smelting, and pesticide usage are all major anthropogenic sources of heavy metals. Lead (Pb), cadmium (Cd), mercury (Hg), and chromium (Cr) are all very dangerous trace elements (Jyothi, 2021). Heavy metals cause acute and chronic toxicity in humans and have been related to cancer, especially in the upper gastrointestinal tract. Heavy metals are picked up by foraging bees from polluted water, air particles, and plants and cling to their body hairs (Zaric et al., 2017). These metals may be found in stored pollen, often known as bee bread, as well as beeswax, honey, and propolis, a resin-like compound obtained from trees after they return to their colonies. Elevated heavy metal levels in bees may have a deleterious influence on brood production, navigation ability, and survival rates (Burden et

al., 2019; Moron et al., 2014). Metal deposition in honey bees and their hives, on the other hand, is usually non-lethal to the colony and provides a chance for environmental monitoring. Conti and Botre (2001) discovered that honey bees and their hive products, pollen, propolis, and wax, were higher in heavy metals in the heart of Rome with substantial vehicle traffic than in places outside the city. Van der Steen et al. (2012) used inductively coupled plasma-atomic emission spectrometry to demonstrate geographical and temporal fluctuation of metal contents in adult honeybees in a 2006 research comprising biweekly sampling of honey bees in three sites in the Netherlands over three months. Ruschioni et al. (2013) examined heavy metal concentrations in honey and entire bees in Italy. Metal pollutants were found in greater concentrations in forager bees than in honey samples, most likely owing to exposure during foraging operations. A lower level of heavy metals observed in honey compared to other bee matrices is consistent with previous research (Alvarez-Ayuso and Abad-Valle, 2017) and suggests that highly precise laboratory equipment is required for analysing honey as a monitoring matrix and quantifying differences between sites (Smith and Weis, 2020). Ruschioni et al. (2013) further showed that metal contamination trends were connected to weather patterns and human activity in the location where samples were collected. Chromium was shown to be the most abundant metal, and the months in which Cr most often surpassed threshold values were associated with a lack of rainfall before sampling. Other

research has demonstrated that rainy weather reduces metal concentrations in honey bees (Zaric et al., 2017). Nickel (Ni) was the least abundant metal, which is consistent with the region's minimal use of coal and fuel oil (Ruschioni et al., 2013). Some heavy metals, such as lead, have numerous isotopes that might be connected to pollution sources. Studies in Serbia, Australia, and Canada have proved the efficacy of utilising honey to monitor trace metal concentrations and Pb isotopic composition at the local scale. Zhou et al., 2018; Zaric et al., 2018). Zaric et al. (2018) used stable isotopes and Kohonen self-organizing maps to investigate spatiotemporal fluctuations and the sources of Pb contamination. Smith et al. (2019) also assessed Pb, Cd, Cr, aluminium (Al), and copper (Cu) contents, as well as Pb isotopic compositions, in honey samples collected from various sectors within the Greater Vancouver Regional District in British Columbia, Canada. When compared to suburban and rural locations, anthropogenically derived trace elements were usually higher in honey from downtown Vancouver. The only exception was manganese (Mn), which was found in the greatest proportions in Delta honey. Manganese is widely present in fertilisers and pesticides, and Delta is a very agricultural region (Smith et al., 2019). When compared to honey from rural regions, honey from hives near Vancouver's major shipping port exhibited greater amounts of trace Pb, higher 208Pb/206Pb isotopic ratios, and lower 207Pb/206Pb isotopic ratios. The port's shipping pollutants were hypothesised to lead to elevated

trace Pb levels and the distinctive Pb isotopic composition of downtown honey. Pb isotopic ratios in honey from rural and suburban regions were comparable to those reported in other environmental proxies, such as oysters and lichens obtained from unpopulated locations on British Columbia's west coast (Smith et al., 2019). Smith et al. (2021) published data from honey samples collected throughout the globe that demonstrated local Pb gradients and Pb isotopic compositions in honey related to human activities in more recent research. A comparison of different matrices in a follow-up study examined variations in trace element levels and Pb isotopic compositions in honey, bee tissues, bee bread, and propolis (Smith and Weis, 2020). Bee tissues and bee bread followed the same pattern of accumulation previously reported for honey by Smith et al. (2019). However, propolis was less effective at mirroring spatial variations in trace metal contamination (Smith and Weis, 2020). Beeswax is another hive matrix that can be used for monitoring metal contamination, in particular long-term exposures. Beeswax has a lipid-based composition that allows for the accumulation of environmental contaminants (Calatayud- Vernich et al., 2017). Gajger et al. (2019) compared the levels of heavy metals in newly constructed hive combs and old reused combs (Fig. 30). Pb levels were higher in reused combs compared to the new combs, and Cu and Pb levels were highest in combs from hives exposed to intensive agricultural or industrial activity (Gajger et al., 2019). Their findings support the biomonitoring capabilities of beeswax,

although data from reused combs may represent recent as well as past colony exposure to contaminants.

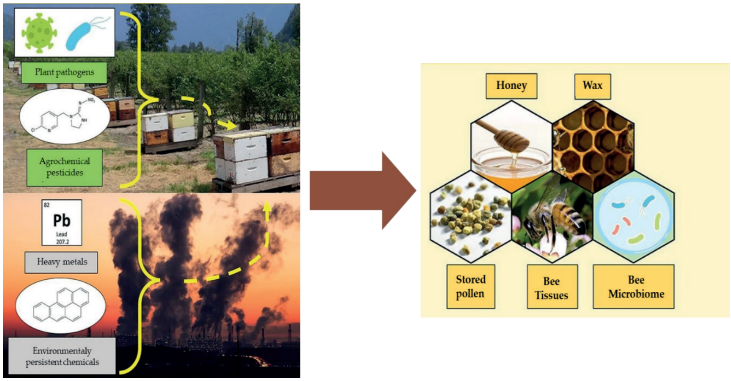

Fig. 30. *Bee products contamination by heavy metals (adopted from Cunningham et al., 2022)*

Heavy metal concentrations, Pb isotopes and molecular biomarkers like AmMT in honey bees and/or their hive matrices align with spatial variations in metal pollution sources. Monitoring heavy metals in the environment regularly could offer insight into the degree and source of pollution with broad implications for protecting human and ecosystem health. Frequent monitoring can be implemented by measuring levels of metals, isotopic ratios and/or relevant biomarkers in honey bees, honey, and beeswax.

Ultrafine particulate matter. Ultrafine particulate matter (UFP), often known as PM with a diameter smaller than 0.1 μg, is a newly recognised pollutant that currently lacks regulatory oversight. Ultrafine particles (UFP) have

the potential to induce pulmonary inflammation and cardiac illness. Additionally, UFP may directly infiltrate the brain via the olfactory bulb, therefore impacting the functioning of the nervous system. In densely populated metropolitan areas, diesel and petrol cars are significant contributors of ultrafine particles (UFP), which include solid particles produced during combustion and particles containing metals (Fig. 31). Metal-based ultrafine particles (UFP) are a subject of significant concern due to their potential to induce inflammation and cause DNA damage via oxidative stress, resulting in the production of free radicals and reactive oxygen species (ROS).

The use of honeybees as an alternative sampling technique for ultrafine particles (UFP) was employed in a region located in the Po Valley in Northern Italy, known for its high levels of vehicular traffic. Worker bees are often acknowledged for their effectiveness in sampling air contaminants, such as airborne particulate matter (PM). The presence of fine hair on the bodies of bees, known as pubescence, plays a role in the buildup of electrical charge during their flying and foraging activities. This electrical charge has been shown to increase the bees' attraction to air contaminants. The bees residing near the main Italian motorway, known as the Autostrada A1, exhibited evidence of contamination by nanosized Fe-oxides/hydroxides and barytes. The main contributors of iron-bearing and barite ultrafine particles are mostly cars that are travelling at high speeds on the motorway. The pollen gathered by forager bees and the honey generated by the bee colony exhibited traces

of contamination from nanosized Fe-oxides/hydroxides and barytes. The presence of such pollution provides a risk to both pollinators and people since it exposes them to the ingestion of ultrafine particles (UFPs). This, in turn, jeopardises the safety of food produced in areas impacted by high levels of traffic (Papa et al., 2021).

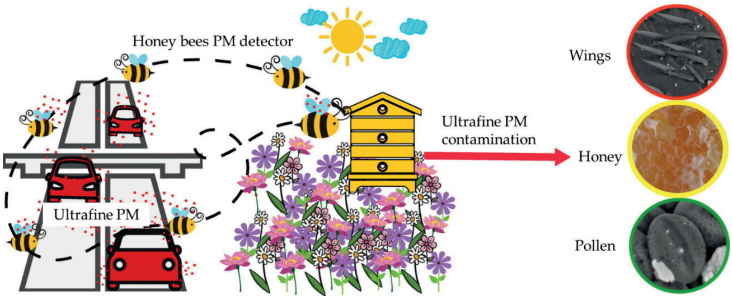

Fig. 31. The ways of ultrafine PM contamination of bee products (source: Papa et al., 2021).

Persistent chemicals and airborne particulate matter. Air pollution caused by environmentally persistent pollutants, particle matter (PM), and other air contaminants is a major worldwide issue that has been linked to respiratory disorders and lung cancer. Environmentally persistent compounds, sometimes known as persistent organic pollutants (POPs), are a worldwide problem. These chemicals may move great distances in the air or water and are resistant to degradation (Wania and MacKay, 1996). Furthermore, there is compelling evidence that these chemicals are bioaccumulated, biomagnified, and transported by migratory species (Montory et al., 2020). These qualities, together with their toxicity,

make it critical to build accurate POP monitoring regimes. We show evidence that honey bees and their hive matrices may be used to test two kinds of POPs: polychlorinated biphenyls and polycyclic aromatic hydrocarbons (Villalba et al., 2020). Polychlorinated biphenyls (PCBs) are a type of ecologically persistent synthetic organochlorine compounds that are discharged via landfills holding obsolete electrical equipment, municipal garbage incineration, and evaporation from polluted lakes. PCBs are a major concern because they accumulate in human tissues and are linked to immune system suppression, as well as an increased risk of cardiovascular disease and cancer (Carpenter, 2006). Sari et al. (2020) investigated the possibility of monitoring PCBs using honey bees and their products. Honey bees were the most polluted with PCBs of the bee-associated matrices studied, followed by honey and bee-collected pollen.

PCBs have limited water solubility and are difficult to incorporate into plant vascular systems, which explains their low quantities in bee pollen. The pollen values most likely indicate PCBs on the surface of pollen (Sari et al., 2020). PCB concentrations in forager bees were greatest during hot, dry weather, as enhanced evaporation of PCBs from contaminated soil exposed them to greater amounts in the air (Sari et al., 2020). Based on a correlation study of PCB levels acquired with PASs and examination of bee-associated materials, Sari et al. (2021) advocated employing bees and honey samples as an alternative to passive air samplers (PASs) for the

monitoring of atmospheric contaminants. Another form of air pollution that occurs naturally in coal, crude oil, and petrol is polycyclic aromatic hydrocarbons (PAHs). Vehicle exhaust, cigarette smoke, wood burning, and asphalt road emissions all emit PAHs into the environment (Centre for Disease Control, 2017). Some PAHs cause eye and lung irritation and are thought to be carcinogenic. Perugini et al. (2009) discovered a variety of PAHs in worker honey bees and honey collected from two separate locations in Italy. Lambert et al. (2012) measured PAH levels in pollen, honey, and bees in French apiaries. They proved that bees were the best markers of PAH pollution in the environment and that the amount of discovered PAHs was impacted by the location in which the apiary was situated. Al-Alam et al. (2019) investigated PAHs in honey samples from Lebanon and showed that PAH sources, such as petrol or vehicle emissions, may be inferred based on honey readings. The PAHs detected in honey corresponded to human activities common in the various locations; population-dense areas had greater levels of PAHs correlating to fuel combustion and vehicle emissions, contrasted to less dense regions, which were dominated by PAHs generated through burning wood for heat.

Negri et al. (2015) investigated the distribution of airborne PM on worker honey bees in a severely contaminated post-mining area in Italy. They discovered airborne PM to be highly concentrated along the margins of the forewings, the medial plane of the head, and the inner surface of the hind legs

using electron microscopy and X-ray spectroscopy (SEM-EDX), all of which contain secreted wax that traps airborne PM. The sorts of particles found in soil and sediment samples from the location matched those found on worker body parts, suggesting that honey bees are excellent pollinators of airborne PM (Negri et al., 2015). Metal-based ultrafine particulate matter (UFP), i.e., PM smaller than 0.1 μg in diameter, has been found in honey bees, bee-collected pollen, and honey collected in a high-traffic location in Northern Italy (Papa et al., 2021).

Microplastics, which are minute bits of plastic generated for industrial reasons or degraded from bigger materials, are another kind of PM (Zhang et al., 2020). Microplastics were mentioned by Amato-Lourengo et al. (2020) as an emerging class of air pollutants with possible implications for human respiratory health. They studied human exposure in metropolitan areas, as well as the physical and chemical properties of airborne plastic waste, the presence of additives, and polymer distributions. Wind often transports the particles, which may be carcinogenic or act as a medium for other environmental toxins (Zhang et al., 2020). Microplastics may cling to the honey bee body and so be quantified, according to Edo et al. (2021). They discovered greater amounts of microplastics in urban locations, whereas suburban and rural levels were similar, owing to wind dispersal.

Agrochemical pesticides. Agrochemical pesticides are persistent toxins in the environment that may harm people,

pollinators, and ecosystems. Because each bee is sensitive, the colony unit is robust, and there are numerous bee-related testable matrices, honey bees and their hives may be utilised as bioindicators of agricultural pesticides (Barganska et al., 2016; de Oliveira et al., 2016). Furthermore, since honey bee foraging preferences coincide with those of other bee species and insect pollinators, evaluating pesticide levels in bee matrices offers useful information on the exposure of other pollinator species in an environment (Bishop et al., 2020).

Neonicotinoids are the most frequently used pesticide class in the world (Simon-Delso et al., 2015). Neonicotinoids, like other pesticides like fungicides and next-generation insecticides, have been shown in lab and field studies to reduce colony survival or have sub-lethal effects on bees, such as impaired memory and foraging activity, as well as reduced immunity (Des Jardins et al., 2021; Tosi et al., 2021; Tsvetkov et al., 2017). Bees are markers of acute pesticide exposure when pesticide levels are fatal. The colony, on the other hand, often survives, and the bees and their related matrices may be employed for short- and long-term monitoring. Traynor et al. (2002) discovered 120 agrochemical active compounds or metabolites in bee-collected pollen. Although most detection levels were projected to provide little damage to honey bee colonies, the research suggested that bee-collected pollen may be used as a terrestrial bioindicator of pesticide exposure. Pesticide quantification in hive matrices is dependent on the chemical properties of the pesticide being tested as well as the

hive matrix employed. Niell et al. (2017) explored how honey bee foragers transmit three neonicotinoids from soybean fields to the colony and assessed the accumulation in three hive matrices: pollen, honey, and beeswax. All three neonicotinoids were found in beeswax, but acetamiprid had the lowest transfer ratio, perhaps because of its high volatility, whereas thiamethoxam had the greatest (Niell et al., 2017). Calatayud-Vernich et al. (2018) examined pesticide concentrations in in-hive bees, newly stored pollen, and beeswax. Beeswax had the highest quantities of agrochemicals, whereas pollen had the largest variety of pesticide kinds. Pesticide concentration in pollen was shown to be greater in intensive agricultural settings compared to rural or grassland settings, showing that pesticides in stored pollen might reflect spatial variations in pesticide contamination in the environment (Calatayud-Vernich et al., 2018). Murcia-Morales et al. (2020) suggested the use of a non-biological in-hive strip that works as a passive pesticide sampler in honey bee colonies as an alternative to sampling bees and bee matrices.

Bees and their colony matrices may be sampled to detect places where chemicals may be harming pollinator health or causing harmful circumstances for human populations. Additional testing using environmental or human urine or blood samples, as employed for the environmental chemical assessment of the Canadian population (Pollock et al., 2021), may also be guided by bee-based monitoring. Furthermore, data collected from testing honey bees and bee-associated

matrices after pesticide pollution episodes might be utilised as an indication of possible pesticide exposure impacting human, animal, and ecosystem health, and could be incorporated as part of the One Health concept (Martinello et al., 2021).

Understanding the potential effects of pesticide exposure on honeybee colonies is crucial since it has been shown that the loss of honeybees inside hives may have a more significant influence on overall colony health compared to the loss of foraging bees. Therefore, it is essential to investigate the extent of pesticide exposure in beehives via the consumption of contaminated substances present within the hive. In this study, a four-year monitoring survey was conducted to analyse 64 pesticide residues in pollen, nectar, and other beehive matrices (such as beebread and honey) obtained from China's primary honey production regions. The analysis was performed using a modified version of the QuEChERS multi-residue technique. The findings indicated that in the samples analysed, a significant proportion of pollen (93.6%), nectar (81.5%), beebread (96.6%), and honey (49.3%) included at least one target pesticide, with concentrations either at or above the method detection limits (MDLs). Furthermore, the analysis revealed the presence of up to 19 different pesticides in each sample. The pesticide that was most often found in the samples, with a presence in over 85% of them, was carbendazim. Additionally, pyrethroids were found in significant quantities, with a median concentration ranging from 134.3 to 279.0 μg/kg. The study demonstrated the occurrence of pesticide transfer from the

surrounding environment to the beehive; nevertheless, it is important to note that the pesticide transference ratio might be influenced by several intricate aspects. While the general danger to the health of colonies due to pesticides seems to be below acceptable limits, the analysis of hazard quotient/ hazard index (HQ/HI) indicates that pyrethroids have a significant impact, contributing up to 45% of the HI value (Fig. 5). In aggregate, these empirical observations provide more understanding of the magnitude of contamination resulting from the use of agricultural pesticides on honeybee colonies (Xiao et al., 2022).

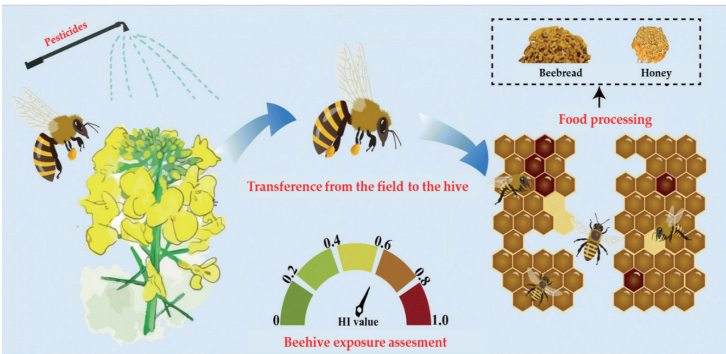

Fig. 32. Honey bee exposure to multiple pesticide residues in the hive environment (source: Xiao et al., 2022)

Pathogenic bacteria. Honey has extremely low water activity, prohibiting bacterial proliferation and, in most circumstances, survival. In addition, relatively few pathogens have been discovered in honey. The presence of Clostridium botulinum in honey, on the other hand, raises

health concerns. This bacterium's spores can live in honey, but they cannot produce the toxin. Ingestion of honey has been linked to baby botulism in a few uncommon instances. As a result, several honey packers (for example, the British Honey Importers and Packers Association) have included a warning on the honey label stating that "honey should not be given to infants under the age of 12 months." On the other hand, this bacterium is often found in natural foods. A European Union scientific commission has investigated the dangers of Clostridium botulinum in honey. It has been determined that no microbiological studies of honey are required since the prevalence of Clostridium botulinum is minimal, and testing will not prevent baby botulism. Only pollen from the other bee products may cause bacterial contamination, therefore bacteriological safety must be controlled.

Genetically modified plants. Genetically modified organisms (GMO), such as rape and maize, are grown in some countries and might pose problems for bees and beekeepers (Williams, 2002a, b). In some countries such as USA and Canada genetically modified plants are commonly grown and accepted by the public, while in the European Union, there is a wide opposition against the consumption of GMO - containing food. In the European Union, the appellation of GMO content in food is compulsory above 1% (EC, 2000b). There are very sensitive methods for the determination of genetically modified plants and pollen. Indeed, the use of polymerase chain reaction (PCR) methods allows the determination of only a few grains

of genetically modified pollen (Ramsay et al., 1999). Bee pollen can be thus significantly contaminated, while honey, which contains less than 0.1% of pollen, will require no specific appellations. **Microplastics** (MPs) are widely distributed and long-lasting contaminants, and have been identified in several environments, ranging from terrestrial to aquatic ecosystems (Fig.6). A recent study has shown the presence of MPs, which mostly include polyethene, polypropylene, and polyacrylamide polymers, in around 12% of honey samples collected in Ecuador. In recent studies, honey bees obtained from apiaries located in Copenhagen, Denmark, along with surrounding semi-urban and rural regions, have been shown to have MPs. The evaluation of the impacts of MP exposure on honey bees is of utmost importance to comprehend the potential dangers associated with such recorded exposures. The honey bee gut microbiome had a loss in diversity because of exposure to polystyrene (PS)-MPs. This was accompanied by alterations in gene expression associated with oxidative damage, detoxification, and immunology. Consequently, the objective of this viewpoint was to examine whether the pervasive occurrence of microplastics (MPs) may potentially have adverse consequences on the well-being and physical condition of honey bees. Additionally, it sought to raise awareness among the scientific community about the potential hazards that MPs pose to the overall fitness of honey bees (Al Naggar et al., 2021).

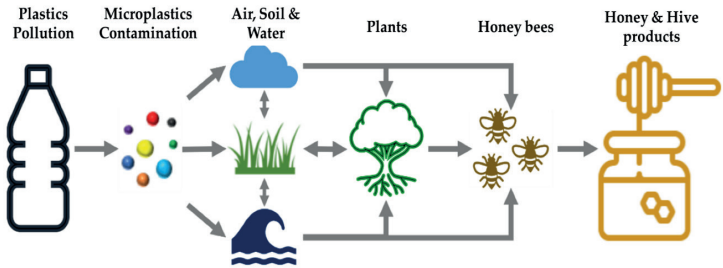

Fig. 33. Microplastic particle mass flow in the environment and potential translation into honey bees and other hive products (source: Al Naggar et al., 2021)

8.2. Indicators of contamination of bee products and the impact of contaminants on the quality and safety of bee products

In modern times, the goal is to make people more aware of environmental pollution. Pollution has been seen to have a direct effect on the health and lives of many different living things. The climate is always changing because of intense farming methods that are needed to make more food as the human population grows (Stepanowski et al, 2010). Pesticides and fertilisers used in these processes may get into food and make it easier for these chemicals to get into different parts of living things. It's important to keep an eye on the world because of this. Even small changes that are bad for an environment can cause bioaccumulation of xenobiotics in the tissues and organs of plants and animals, which can lead to mutations and diseases. Honey bees (*Apis mellifera*) depend on the health of the surroundings for their survival. In addition to a diverse

array of commercial crops, honey bees play a crucial role in the pollination of several wild plants, including some that face the risk of extinction and possess significant genetic value (Fig. 6). In recent years, honey bee populations have shown a generally stable trend with occasional fluctuations, indicating a modest increase in numbers.

However, it is crucial to acknowledge the presence of several challenges that significantly impact the health and survival of honey bees. The important variables identified in this study are the presence of inadequate nutrition, exposure to sublethal levels of insecticides, and the influence of biotic stressors like illnesses and parasites. The decrease in honey bee numbers has adverse effects on both commercial agricultural and flowering plant populations. Additionally, it diminishes the availability of several honey bee products, including honey, bee pollen, propolis, bee venom, royal jelly, and beeswax. These products have been recognised for their major contributions to human health benefits. Numerous ideas have been proposed in an endeavour to elucidate these declines; nevertheless, so far, no unequivocal cause has been ascertained as the primary catalyst for the reduction in bee populations. Therefore, it is essential to promptly implement strategies aimed at safeguarding and conserving the honey bee population by identifying and mitigating the primary factors contributing to this occurrence. Various conservation measures have been used to safeguard the populations of *A. mellifera* and honey bees in significant regions of the globe.

Nevertheless, the study also highlights the discrepancy in the allocation of resources and information between honey bees and other pollinators (Xiao et al., 2022).

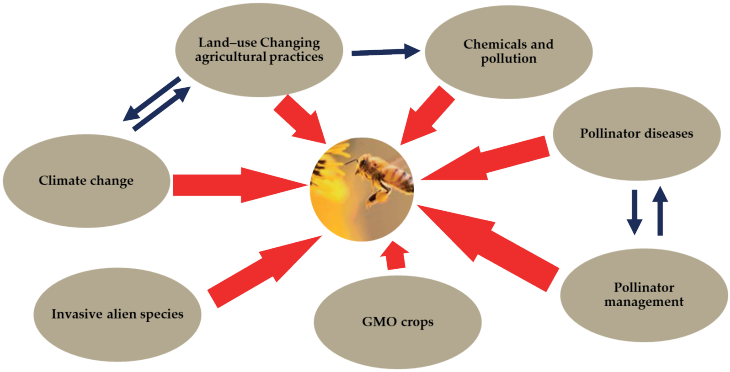

Fig. 34. Main drivers of change of honey bee colony declines (adopted from Xiao et al., 2022).

During the active phase, honey bees gather nectar, honeydew, and flower pollen from which they manufacture bee products. Flower pollen, bee bread, honey, and propolis are examples of vegetable-derived goods (Cichocki et al, 2000). The other is made up of bee products (such as royal jelly, beeswax, and bee venom). From spring until fall, honey bees are continually exposed to the activity of pollutants located in the neighbourhood of the hives, a region that may be as vast as 7 km^2. When foraging, bees bring pollutants from their meal as well as those deposited on the surfaces of the flowers and leaves of the plants they visit back to the hive (Fig. 7). The xenobiotics stick to the bees' bodies and enter them via the air they breathe (Porrini et al, 2002).

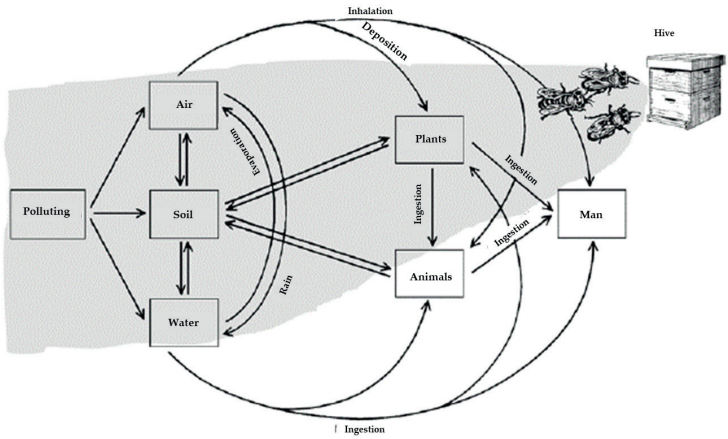

Fig. 35. The diffusion of polluting substances in the environment (the grey area shows the environmental sectors visited by the honey bees) (source: Porrini et al, 2003)

These xenobiotics may enter the hive via many different paths and methods. Honey bees and the products they produce may be polluted directly by beekeeping practices as well as indirectly by toxins from agricultural practices and the environment in general (Kujawski, Namiesnik, 2008). *Varroa jacobsoni* is a parasitic mite that harms honey bee colonies. Some insecticides, like coumaphos and malathion, are used to combat it (Fernandez et al. 2001). One of the most dangerous parasites to honey bees is the *Varroa* mite, also known as *Varroa destructor*, which may harm both adult bees and young bees (Calderon et al., 2009). The first synthetic varroacides used was pyrethroid tau-fluvalinate, a subset of fluvalinate isomers, which was sprayed on plywood strips strung between

brood frames. As tau-fluvalinate's effectiveness against *Varroa* started to fade, varroacides such as coumaphos (an organic phosphate pesticide), amitraz (a formamidine pesticide), and fenpyroximate (a pyrazole acaricide) were launched (Elzen, 2000). Today's synthetic varroacides are often lipophilic and may persist in the wax component of hives for years after application.

Honey bee deaths are mostly caused by pesticide residues, and the presence of pesticides and other contaminants like heavy metals and radionuclides in their bodies or beehive products, which can be found with the right lab tests (Conti et al., 2001). Evaluating the amount of xenobiotics in bees and their products is done not only to figure out how good these things are but also to figure out how polluted the world is as a whole (Fig. 8).

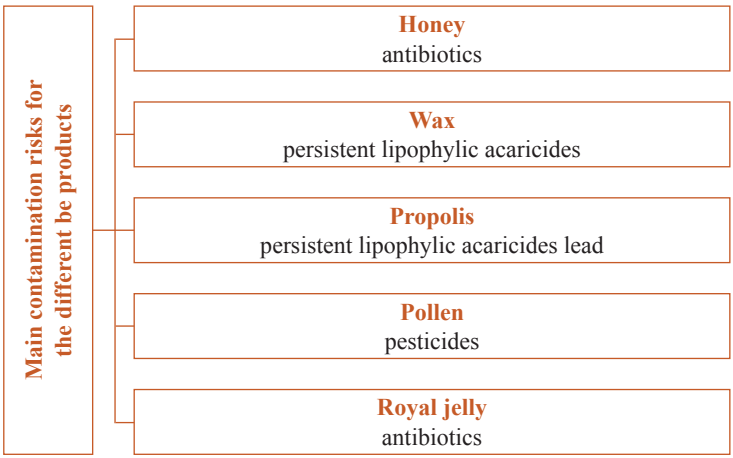

Fig. 36. The main contamination risks for the different bee products

Beekeepers can take successful measures to prevent the contamination of bee products from beekeeping sources, as in all cases there are ecological alternatives. A HACCP (Hazard Analysis and Critical Control Point) system for the control of contamination sources should be developed and applied to beekeeping. The present review provides the foundations for the establishment of such a system, as it covers all major bee product contaminants. Alternative bee pest control strategies and minimal use of synthetic chemicals in beekeeping can keep bee products clean and safe. The introduction of organic beekeeping is an ecological means to avoid all major contamination sources for the production of high-quality bee products, free of toxic contaminants (Bogdanov, 2005).

Pesticides are toxic to bees mostly because of the active ingredient (LD_{50}), the presence and length of bloom on cultivated or wild plants, the presence of honey bees on the site and at the time of chemical treatment, the way the pesticide is spread, and the wind. When bees come into touch with poison, many of them don't go back to the hive and die in the field or on their way back. The other bees will eventually die in the hive, which will be a clear sign. Honeybees work as a secondary signal and tell us about the residues they were subjected to (Shrestha, 2004). This is true for chemicals that are not very dangerous.

Heavy metals present in the atmosphere can deposit on the hairy bodies of bees and be brought back to the hive with pollen, or they may be absorbed together with the nectar of the

flowers, or through the water or the honeydew. Many variables have to be considered when using bees, or beehive products such as honey, to monitor heavy metals in the environment: the weather (rain and wind can clean the atmosphere or transfer heavy metals to other environmental sectors), the season (the nectar flow, which is usually greater in spring than in summer and autumn, could dilute the pollutant), the botanical origin of the honey (the nectar of flowers with an open morphology and the honeydew are much more exposed to pollutants). 43 samples of honey bees from 16 hives and 74 samples of honey from 29 hives were examined in the same conditions and using the same procedures. The statistical analyses showed a slightly higher degree of reliability for honey, statistically significant only for chromium. To better investigate the matrix honey bees, were analysed 178 samples of foragers, caught on their return to their hives in three different areas: urban, industrial and natural. The amount of metal accumulated inside the bee and deposited on the bee surface was analysed. The lead in the urban and industrial areas was found in higher quantities inside the bee than on the bee surface, to a highly significant degree ($p<0.0001$) while the ratio was inverted in the natural areas ($p<0.0005$). Concerning nickel, a significant difference was found only in the natural area ($p<0.05$), where the amount was again higher on the bee surface. For chromium a significantly higher amount was found on the bee surface in all three environments (urban: $p<0.05$; industrial: $p<0.005$; natural: $p<0.005$). The results for lead could indicate that persistent

contamination induces higher absorption of pollutants, by inhalation or ingestion, into bee bodies during foraging. The higher levels of lead, nickel and chromium metals on the bee surface in natural areas could suggest that the pollutants are scattered throughout the atmosphere and they do not impregnate or deposit on the environmental components visited by bees. Nickel and chromium differ from lead in the two most highly contaminated areas. This discrepancy is probably due to their different environmental fate. However, it also reflects the high number of cases in which the values recorded inside and on the bee were equal because they were below the limit detectable by the instrument (Porrini et al 2003)

Investigations of honey bees and hive products regarding radioactivity date back to the end of the 1950s, but it was not until the Chornobyl state of emergency (April - May 1986) that the excellent efficacy of bees in detecting radioisotopes was unequivocally demonstrated. In a research project carried out by our team, again in the context of Chornobyl, numerous samples of honey bees, wax and pollen were analysed. The findings demonstrated that pollen was the most efficient indicator of atmospheric radionuclide contamination. Towards the end of April 1998, an incident occurred at the Algeciras steel works in southern Spain with emissions of caesium 137, coming from a radioactive source no longer in use. In May 1998, our radiochemical laboratory detected an anomalous presence of 137Cs in honey bee samples taken from monitoring stations in the Bologna province. The hypothesis

that the anomalous radioactivity derived from active nuclear plants can be ruled out, as the 137Cs were not accompanied by the other radionuclides produced during fission. The fact that the presence of 137Cs was interrupted for a week and then resumed is not unusual as the transport and soil deposition of air-dispersed pollutants is strictly linked to wind and precipitation. The levels of radioactivity were negligible and many times below every alarm threshold, but the bee matrix promptly revealed the presence, albeit minimal, of 137Cs in the atmosphere with efficiency above that of traditional monitoring techniques.

Threats to human health resulting from the use of contaminated bee products

According to European Union regulations, honey as a natural product must be free of chemicals (Directive 2014/63/EU of the European Parliament and of the Council). Poisoning bee pollinators is a substantial negative impact of pesticide usage, resulting in a decline in the insect population, a decrease in honey output, the destruction of plant communities, the presence of insecticide residues in food, and, eventually, a considerable loss of a beekeeper's revenue. The primary goals of monitoring bee products are to safeguard consumer health, increase worldwide commercial competitiveness, and improve product quality.

Pesticides. Pesticides are utilised globally to combat bee illnesses and pests, and in most cases, their administration

is unregulated and without recognised methods. Pesticides are used to protect crops and boost agricultural output. Uncontrolled applications, on the other hand, may contaminate the environment, animal species, and humans. The systematic introduction of pesticides into nectar and pollen may have a direct impact on honey bee health and eventually lead to pesticide contamination of honey-containing meals. Pesticides are hazardous to human health because of their toxicity and the time and quantity of exposure (Lorenz, 2009). Unfortunately, farm labourers and their families are most vulnerable to agricultural chemicals. Because of their tiny stature and underdevelopment, children are the most vulnerable and sensitive to pesticides. Importantly, the compounds may bioaccumulate, biomagnify, and bioconcentrate in the body over time. Pesticide exposure may cause anything from moderate skin irritation to birth deformities, cancers, genetic alterations, blood and nerve diseases, endocrine disruption, and even death. Persistent organic pollutants (POPs) include Aldrin, chlordane, DDT, dihedron, endrin, heptachlor, hexachlorobenzene, mirex, and toxaphene (Ritter et al., 2010). POPs have the potential to harm the endocrine, reproductive, and immunological systems. Chronic exposure may cause a variety of ailments, including cancer, neurobehavioral problems, infertility, and mutagenesis consequences. As a result, certain POPs have been outlawed, while others are still in use (Lim et al., 2010).

Antibiotics. Consumers are increasingly concerned about antibiotic residues in honey. Some medications have the

potential to cause hazardous responses in consumers, while others have the potential to cause allergic or hypersensitive reactions. At extremely low dosages, lactam antibiotics produce cutaneous eruptions, dermatitis, gastrointestinal problems, and anaphylaxis (Diserens, 2007). Microbiological risks, carcinogenicity, reproductive impacts, and teratogenicity are all long-term consequences of antibiotic residue exposure. Microbiological impacts are one of the most serious health issues in humans. Certain medicines, such as nitrofurans and nitroimidazoles, have been linked to cancer in humans. Similarly, even at extremely low dosages, certain medications may have reproductive and teratogenic consequences. Bacterial populations may grow resistant to antibiotic residues present in food and honey. Antibiotic resistance is a global public health issue that has proven difficult to solve. Antibiotic resistance has been listed as one of the top three dangers to human health by the WHO. The main cause is long-term exposure to antibiotics because of their use as drugs in humans and animals, in horticulture, and in food preservation. Animal antibiotics are often the same as human antibiotics. More data suggests a link between antibiotic usage in food animals and antibiotic resistance in bacteria isolated from people. A pig farm was linked to an epidemic of human nalidixic acid-resistant Salmonella typhimurium DT104 infection in Denmark. Another incidence of the same virus was recorded in the United Kingdom, and it was tracked back to a dairy farm where fluoroquinolones were used on the cattle

a month before the outbreak. Following the first authorized use of fluoroquinolones in food animals in 1995, there was a significant increase in the percentage of fluoroquinolone-resistant *Campylobacter* infections in the United States.

Antibiotics approved for human use should not be used as growth boosters in cattle, according to the WHO. Since then, research from Denmark, Germany, and Italy have shown a considerable decrease in vancomycin-resistant *Enterococci* isolations from chicken and poultry-derived food items. Regardless of their relevance to human health, certain European member states have voluntarily stopped the use of all growth boosters.

Bee pollen is a popular nutritional supplement, although there are no rules in most countries. As a result, these goods may provide a variety of food safety issues. Pesticides, heavy metals, metalloids, and mycotoxins are common pollutants in bee pollen. These items may also include pyrrolizidine alkaloids, allergenic proteins, and pollen grains from genetically engineered plants. Recent studies on the compounds, as well as data on amounts discovered in bee pollen, are described in this study. Based on the literature, a risk assessment was also completed for the toxicologically relevant pollutants and elements of bee pollen. Our findings imply that pesticides routinely detected in pollen do not endanger human health. Pollen loads, on the other hand, maybe polluted with metals, metalloids, and mycotoxins to the point where they pose a concern to consumers. Certain

plant species have unusually high levels of hepatotoxic pyrrolizidine alkaloids; accordingly, bee pollen intended for human consumption should be monitored. The number of scientific research on the topic of bee pollen-associated food safety risks has continuously increased in the last two decades, but the information is incomplete in some areas. In Europe, many studies are conducted, but little data is available from other continents (Végh, 2021). Honey is a natural substance that is commonly utilized for nutritional as well as therapeutic reasons. Honey, like other foods, is vulnerable to infection and adulteration. Markets are brimming with unlabelled and tainted honey. Pesticides, herbicides, antibiotics, and heavy metals are among the microbial and non microbial pollutants found in honey samples from throughout the globe. As a result, ingesting it without knowing where it came from or how safe it was might pose serious health risks. Honey labels must be substantiated by analysis confirming their origin and safety. To ensure the safety of honey, health authorities in all countries must enact strict regulations and rules that govern and regulate honey production, handling, and analysis. Raw honey that has not been tested or sterilized should not be given to newborns. Furthermore, raw honey should not be administered to wounds or lesions without first being sterilized, and it should be analyzed to detect any adulteration that may influence its medicinal effects. These suggestions should also be addressed while using additional bee products as nutritional supplements or medical therapies,

such as wax, bee venom, pollen, and royal jelly. Because residual amounts of pollutants cannot be modified using different manufacturing procedures, proper monitoring is essential. Market rivalry for these items imposes additional requirements that can only be met by adhering to quality assurance and certification processes and regulations.

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Standardization and Certification of Bee Products (PL/COMU)

Doç. Dr. Barbara Król *Dr. Maja Słupczyńska*
Uniwersytet Przyrodniczy we Wrocławiu - Poland

Kemal ÇELİK
Çanakkale Onsekiz Mart University- Çanakkale Türkiye

In this module you will learn about specific criteria, guidelines, and regulations to ensure the quality, safety, and consistency of bee products, such as honey, beeswax, royal jelly, and propolis, as key factors for the standardization of bee-derived products what is essential for the protection of consumers, the promotion of fair trade, and the quality control.

STANDARDIZATION OF BEE-DERIVED PRODUCTS

A set of specific criteria, guidelines, and regulations to ensure the quality, safety, and consistency of these products. Standardization is essential for the protection of consumers, the promotion of fair trade, and the quality control of bee-derived products. The specific standards and regulations for bee-derived products can vary by country and region, but

they are generally intended to protect both consumers and producers while promoting the integrity of these valuable natural products. Compliance with established standards can help consumers make informed choices and build trust in the market for bee-derived products.

Quality (composition criteria) standards

Labeling requirements

Traceability

Bee-derived products certification

QUALITY STANDARDS:

The quality parameters that bee-derived products must meet. The quality criteria depends on type of bee-derived product. These criteria help maintain a consistent and high-quality product. Quality standards for bee-derived products, such as honey, beeswax, royal jelly, and propolis, are established to ensure the safety, authenticity, and quality of these products. These standards can vary by country or region, but there are some common quality criteria that are typically applied to bee products. Composition and quality requirements are clearly defined in international standards such as the Codex Alimentarius, The European Directive, the International Organization for Standardization (ISO), the USP Identity Standard for Honey, Turkish Food Codex notification on honey and guidelines of different trade and beekeeping associations.

HONEY QUALITY STANDARDS

The Codex Alimentarius Commission has established a specific standard for honey – revised CODEX STAN 12-1981 (2001) that provides guidelines for the quality, labeling, and packaging of honey. It covers aspects like moisture content, flavor, color, contaminants, labeling requirements, and more. CODEX STAN 12-1981 provides standards for all honeys produced by honeybees intended both for direct consumption and for industrial uses or as an ingredient in other foods. The key legal act on standards for honey in European Union is European directive 2001/110/EC relating to honey. In accordance with the Codex recommendations, the European Council promulgated Directive 2001/110/EC (EC, 2001), subsequently revised 2014/63/EU (EU, 2014), which established the guidelines for honey production and trade among EU member states (EU, 2011, 2014). Click on the one of the below flag icons to make you familiar with European directive 2001/110/EC relating to honey in English and project partner languages (there is no Turkish translation of the Directive). STANDARDS revised CODEX STAN 12-1981 (2001) vs. European directive 2001/110/EC relating to honey.

HONEY QUALITY (COMPOSITION CIRTERIA) STANDARDS revised CODEX STAN 12-1981 (2001) vs. European directive 2001/110/EC relating to honey.

Differences exist only in the provision regarding honey with a natural low content of enzymes and baker's honey.

Honey definition and types – blossom or nectare honey is the honey which comes from nectars of plants, while honeydew honey is the honey which comes mainly from excretions of plant sucking insects (Hemiptera) on the living parts of plants or secretions of living parts of plants. Moisture content - to prevent fermentation a maximum moisture content, not more than 20%. Heather honey (Calluna) not more than 23%. STANDARDS revised CODEX STAN 12-1981 (2001) vs. European directive 2001/110/EC relating to honey.

HONEY QUALITY (COMPOSITION CIRTERIA) STANDARDS revised CODEX STAN 12-1981 (2001) vs. European directive 2001/110/EC relating to honey.

Sugars content fructose and glucose content - not less than 60%, while honeydew honey, and its blends with blossom honey – not less than 45%. sucrose content – for most of honey types not more than 5%, for some types of honey such as alfalfa (Medicago sativa), false acacia (Robinia pseudoacacia) 10%, and for lavender (Lavandula spp), borage (Borago officinalis) – not more than 15%. STANDARDS revised CODEX STAN 12-1981 (2001) vs. European directive 2001/110/EC relating to honey. Color and appearance - depending on the type of honey should meet specific color and clarity criteria. Colour ranges from nearly colorless to dark browns. The consistency can be fluid, viscous or partly to entirely crystallized. Flavor and aroma - flavor and aroma of honey should be associated with its botanical and/or geographical origin. Honey shall

not have any objectionable matter, flavor, aroma, or taint absorbed from foreign matter during its processing and storage. Free from contamination, presence of contaminants, such as antibiotics, pesticides, and heavy metals in honey is not allowed. Pollen analysis - to confirm its botanical origin some standards require the identification and quantification of pollen in honey. Quality standards (composition criteria) of honey (source: Thrasyvoulou A. et al. 2018).

Composition criteria	Directive 2001/110 EU			Revised CODEX 2011
	Blossom honey		Honeydew honey* general	
	General	Exceptions		
Moisture; %	<20	*Calluna* and baker's honey <23; baker's honey from *Calluna* <25	<20	The same. No indication for baker's honey.
Fructose+ glucose; %	>60	-	>45	The same.
Sucrose; %	<5	robinia, medicago, banksia, hedysarum, eucalyptus, *Eucryphia spp*, and citrus <10; lavandula, borago <15	<5	The same.
Water-insoluble; %	<0,1		<0,1	The same.

Composition criteria	Directive 2001/110 EU			Revised CODEX 2011
	Blossom honey		Honeydew honey* general	
	General	Exceptions		
Electrical conductivity; mS/cm	<0,8	chestnut, arbutus, erica, eucalyptus, tilia, calluna, manuka, melaleuca	<0,8	The same.
Free acid; meq/kg	<50	baker's honey <80	<50	The same.
Diastase activity; DN**	>8	baker's honey and honey with low natural enzyme content: >3 when HMF is less than 15 mg/kg	>8	Honeys with low natural enzyme content: > 3 DN.
HMF; mg/kg**	<40	baker's honey honeys of tropical climate and blends of these honey <80	<40	Honeys of tropical climate and blends: < 80.

Table 14. Quality standards (composition criteria) of honey

DISPARITIES BETWEEN EUROPEAN DIRECTIVES, CODEX, AND NATIONAL LAWS

POLAND

Countries with fully harmonized national quality standards (composition criteria) with EU legislation.

TÜRKİYE

Provisions: electrical conductivity mS/cm−1 0.2–0.6 fl, 0.6–0.8 fl+hd (natural mixed),> 0.8 hd deciduous trees,>0.95 hd coniferous; proline >250 mg/kg; HMF <30 mg/kg; acidity >10–50 meq/kg

BEESWAX QUALITY STANDARDS

The process of creating and upholding particular quality standards and requirements for beeswax goods in order to guarantee consistency, quality, and safety is known as "beeswax standardization." For a number of businesses that use beeswax, including food, medicine, cosmetics, and candle manufacturing, standardization is crucial.

The quality standards for beeswax are provided by FAO (2005) as set of guidelines and Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council Text with EEA relevance – (click on the below icons to make you familiar with the detailed information in English about quality parameters of beeswax).

Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council Text with EEA relevance is available in project partner languages, except in

Turkish (click on the below icons to make you familiar with the text of legal act in your mother language)

The age of the wax, the type of bees, and the climate where it is produced all have an impact on the composition of beeswax. Physical-chemical characteristics like melting point, density, acid value, saponification value, ester value, iodine adsorption number, and peroxide value can be used to assess the validity of beeswax. European Food Safety Authority (EFSA, 2020) recommended at least two physico-chemical parameters accompanied with advanced analytical methods to test beeswax purity and quantification of beeswax adulterants (to see the technical report click the below icon).

Purity - high-quality beeswax should be pure, with minimal impurities that can include bits of honey, propolis, and debris from a hive.

Color - ranges from pale yellow to dark brown (depends on the type of flowers the bees forage on)

Flavour - pleasant, mild honey-like scent (rancid unpleasant odor indicates contamination or poor quality)

Melting point- around 62-65°C (143-149°F). This melting point can vary slightly depending on the source of the beeswax.

Texture - smooth and uniform, free from graininess

Impurities - free from foreign materials, e.g. synthetic additives, pesticides, molds, fungal or bacterial contamination.

Crude ash content – low Acidity - neutral pH, typically around 7.0.

Parameter	FAO (2005)	231/2012/EC (2012)	IHC (2016)
Moisture; %	-	-	**Not more than 1%**
Melting range; ^{o}C	62 - 65	62 - 65	61 - 65
Specific gravity; D_{2020}	-	~0,96	-
Refractive index; 75 ^{o}C	-	-	1,4398 - 1,4451
Solubility	insoluble in water, sparingly soluble in alcohol, very soluble in ether	insoluble in water, sparingly soluble in alcohol, very soluble in chloroform and ether	
Acid value; mg KOH/g	17 - 24	17 - 24	17 - 22
Saponification value; mg KOH/g	87 - 104	87 - 104	87 – 102
Ester value; mg KOH/g	-	-	70 - 90
Ester/acid ratio	-	-	3,3 – 4,3
Peroxide value (mM H_2O_2/kg)	Not more than 5	Not more than 5	

Parameter	FAO (2005)	231/2012/EC (2012)	IHC (2016)
Glycerol and other polyols	**Not more than 0,5 % (as glycerol)**	**Not more than 0,5 % (as glycerol)**	**Absent**
Carnuba wax	Test*	No information	Absent
Ceresin, paraffins and other waxes	Test*	Test*	Absent
Fats, Japan wax, resin and soaps	Test*	Test*	Absent
Arsenic	-	Not more than 3 mg/kg	-
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg	-
Mercury	-	Not more than 1 mg/kg	-

Table 15. (source: Bogdanov, 2016)

To learn more about the chemical tests for the presence of individual undesirable compounds click below icons (FAO, 2005 pp.12-13; 231/2012/EC, 2012 pp. 251)

PARAFFIN - most widely used due to its low price, availability, and physico-chemical properties - chemically inert, colorless and odorless.

STEARIN/STEARIC ACID

PALMITIN

TALLOW

Pesticides and veterinary drug residues – recommended limits in beeswax (source: FAFSC (2018)

CONTAMINANTS	LIMITS
Acrinathrin	< 0.6 mg/kg
Amitraz	< 400 mg/kg
Carbofuran	< 0.4 mg/kg
Chlorpyrifos(-ethyl)	< 2 mg/kg
Coumaphos	< 40 mg/kg
Cyfluthrin	< 0.06 mg/kg
Cypermethrin	0.3 mg/kg
DDE	< 40 mg/kg
DDT	< 40 mg/kg
Deltamethrin	< 0.1 mg/kg
Flumethrin	< 1.5 mg/kg
Imidacloprid	< 0.03 mg/kg
Lindane	< 0.09 mg/kg
Mevinphos	< 0.2 mg/kg
Pyridaben	< 1.5 mg/kg
Tau-fluvalinate	< 20 mg/kg
Thiamethoxam	< 0.04 mg/kg
Thymol	< 2 mg/kg

Table 16. Pesticides and veterinary drug residues – recommended limits in beeswax

BEE VENOM QUALITY STANDARDS

Since bee venom is not recognized as an official drug or food, there are no official quality standards for it.

Bee venom as a fresh basis should be a clear, odorless, watery liquid (~ 88% of moisture) when dried – light yellow powder.

The chemical purity may be assessed as a quantitative analysis of more stable or easily measurable components of bee venom, primarily two proteins: melittin (~50% of DM), and phospholipase A2 (10-12%).

The Food and Drug Administration (FDA) of the United States states that venom preparation makers must provide evidence of enzymatic activity:

The hyaluronidase enzyme must be present and exhibit enzymatic activity expressed in units per milliliter of solution (usually, the range is between 50 and 130 U/mL); phospholipase activity must be present, however it is determined with a straightforward plus/minus test.

Analytical procedures to determine bee venom components

Fig. 37. Quality standarts of bee-derived products

POLLEN QUALITY STANDARDS

Official international pollen standards do not exist.

A standard for pollen quality has been proposed by the Swiss Food Manual (2003), which includes composition criteria of quality standards for:

Protein, lipid, carbohydrates, crude fiber, minerals, and vitamins.

The best method to preserve nutritional value and the optimal biological value of pollen, is freezing of fresh pollen under nitrogen. Most often pollen is drired. This proces should be carried out at the temperature not higher than 40℃ (to avoid losses of volatile compounds) untill the mositure content will be lower than 6%.

Analysis	Quality criteria
Sensory parameters	No visible contaminants, typicsl flavour and taste
Microscopic examination	Origin test (geographical, botanical)
Microbial examination	Bacterial load should be within legal hygienic limits
Chemical examination	Moisture content not more than t6% of DM
Contamination	Heavy metals, pesticides*

Table 17. Quality standards of pollen (source: Swiss Food Manual, 2003).

COMPONENT	CONTENT (% of DM)	
	minimum	maximum
Carbohydrates	13	55
Protein	10	40
Ether extract	1	10
Dietary fibre	0,3	20
Minerals	0,05 0,3	0,3
Vitamins	0,002	0,01
Flavonoid glycosides	0,004	0,3

Table 18. Pollen quality criteria (source: Swiss Food Manual, 2003)

ROYAL JELLY QUALITY STANDARDS

Physical parameters	Quality criteria
Consistency	semi-fluid, homogeneous, gelatinous substance
Colour	whitish or beige
Taste	acidic
Flavour	pungent, phenolic
Density	1,1 g/cm^3

Table 19. Royal jellly specification is described in ISO 12824:2016 standard

ROYAL JELLY QUALITY (COMPONENT CRITERIA) STANDARDS (source: ISO 12824:2016 standard)

COMPONENT	Unit	CONTENT	
		minimum	maximum
Moisture	% of fresh material	62,0	63,5
0-hydroxy-2-decenoic acid (10-HDA)		1,4	
Protein		11	18
Total sugar		7	18
Fructose		2	9
Glucose		2	9
Sucrose		<3,0	
Erlose		<0,5	
Maltose		<1,5	
Maltotriose		<0,5	
Total lipid		2	8
Total acidity [1mol/l NaOH]	ml/100g	30	53

Table 20. Royal jelly component criteria

ROYAL JELLY QUALITY STANDARDS – MICROBIAL STANDARDS (source: ISO 12824:2016 standard)

Microorganism	Unit	Limits	Analytical reference method
Colony count Pathogenic bacteria	CFU*/g	< 500	ISO 4833-1
Enterobacteriaceae	CFU/g	0/10g	ISO 21528-2
Salmonella	CFU/g	0/25g	ISO 6579

Table 21. Royal jelly microbial standards

One of the most relevant quality indicators for routine testing of royal jelly authenticity is the content of 10-hydroxy-2-decenoic acid (10-HDA) called queen bee acid. The optional quality parameter determining the freshness of royal jelly is furosine, an indicator of chemical alteration referred to exposure to high temperatures and time.

Very important indicators of royal jelly quality testing are the stable isotopes of the elements carbon and nitrogen in order to detect adulteration with sugar syrups.

PROPOLIS QUALITY STANDARDS

Depending on geographical region, season, forage, and method of extraction, more than 800 different phytoconstituents in various concentrations have been recognized in propolis and propolis extract samples. Unfortunately, there are no available literature reports demonstrating whether the specific therapeutic potential of propolis is associated with a certain chemical entity. Therefore, there is still a need to adapt a more detailed quality control strategy for propolis standardization.

There are two main types of propolis traded internationally – brown (Populus), green (Baccharis), In scientific literature, the most wideły reported types of propolis, besides two mentioned above, is also red propolis. This standard considers the complex chemical composition of propolis, and the influences geographical and plant species variation, and honey bee sub-species have on the proximate, flavonoid, and phenolic composition of propolis.

Propolis from different geographical regions demonstrates considerable biological activity even though the chemical composition may vary. Prior to the analysis, the chemical type of propolis should be determined. The specific approach and requirements for propolis from well-known geographic origins, where it has been demonstrated throughout time to be of constant plant origin, can be applied by default. However, reports on propolis types in the Middle East, Africa , and Australia, are scarce and prove miscellaneous chemistry. Therefore, it is hard to formulate propolis types for these regions.

Recommended analytical methods of dereplication of propolis types and known bioactive metabolites botanical and other natural sources is GC-MS

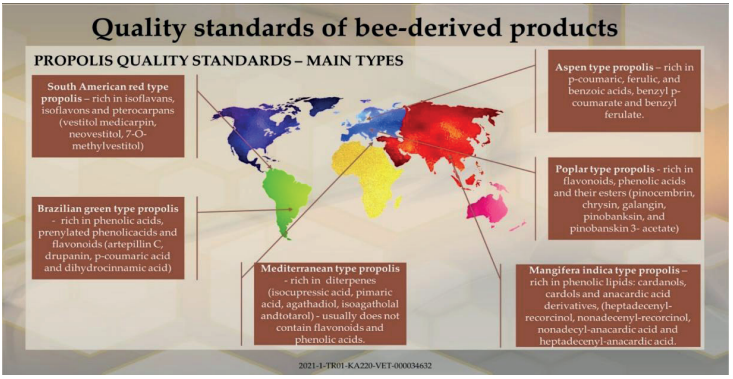

Fig. 38. Quality standards of bee-derived products

PROPOLIS QUALITY STANDARDS FOR ALL TYPES OF PROPOLIS* (source: IHS)

Parameter	Value
Content of matter soluble in 70% ethanol (balsam content)	Not less than 45%
Wax content	Not more than 40% *(Stan et al., 2011)*
Water content	Not more than 8%
Mechanical impurities	Not more than 6%
Ash content	Not more than 5%

Table 22. Propolis quality standards for all types

* Brazilian legislation for Brazilian green propolis recommends a minimum of 35% ethanol extractable substances and a maximum of 25% wax.

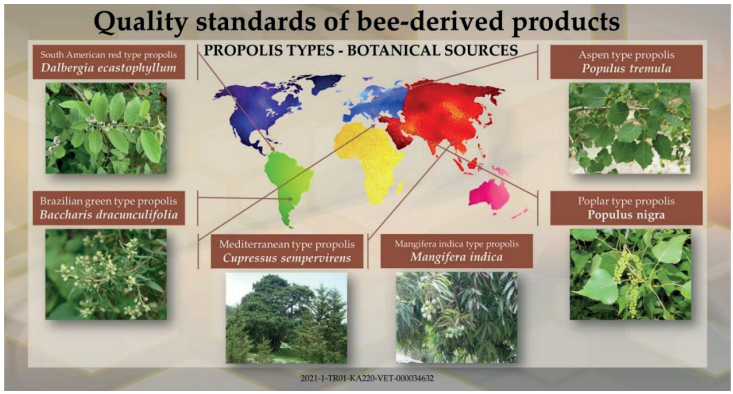

Fig. 39. Quality standards of bee-derived products

The International Honey Commission (IHC) recommends the values for the concentration of biologically active constituents for the two most wide-spread propolis types i.e. European poplar type propolis- Poplar type, and Brazilian green propolis- Baccharis type (source: Bankova et al., 2016).

Propolis type	Bioactive component	Minimum % of raw propolis	Reference
Poplar propolis	Total phenolics	21	(Popova et al., 2004)
	Total flavones and flavonols	4	(Popova et al., 2004)
	Total flavanones and dihydroflavonols	4	(Popova et al., 2004)
Brasilian green propolis	Total phenolics	5	(Sawaya et al., 2011)
	Total flavonoids	0,5	(Sawaya et al., 2011)

Table 23. Recommendations of IHC on the values for the concentration of biologically active constituents for the two most wide-spread propolis types

Bee-derived product certifications

TYPES OF CERTIFICATIONS

ORGANIC CERTIFICATION

PROTECTED DESIGNATION OF ORIGIN (PDO)

PROTECTED GEOGRAPHICAL INDICATION (PGI)

TRADITIONAL SPECIALITY GUARANTEED (TSG)

QUALITY/SAFETY STANDARDS CERTIFICATION

NON-GMO CERTIFICATION

ORGANIC CERTIFICATION

Organic certification of bee-derived products, such as honey, beeswax, and propolis, involves ensuring that these products are produced in accordance with organic farming and processing standards. Organic beekeeping practices aim to minimize the use of synthetic chemicals and promote sustainable and environmentally friendly methods. To achieve organic certification for bee-derived products, producers and beekeepers must adhere to specific guidelines and standards, which may vary by country or certifying body. In the EU the standards of organic beekeeping are described in Regulation 848/2018 on organic production and labeling of organic products (click the icon below).

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ORGANIC CERTIFICATION – STANDARDS

APIARY LOCATION AND MANAGEMENT - organic beekeeping often requires that the apiary is located in an area where bees can forage on organic flowering plants and crops. Bees should not be exposed to synthetic pesticides or genetically modified crops. For organic beekeeping, preference shall be given to the use of Apis mellifera and their local ecotypes.

HIVE MATERIALS - the materials used in beehives, such as wooden frames and foundation, should meet organic standards and not be treated with synthetic chemicals.

BEE FEEDING - organic beekeeping emphasizes the use of organic feed for bees when necessary. This feed should be free from synthetic chemicals and genetically modified organisms (GMOs). At the end of the production season hives

shall be left with sufficient reserves of honey and pollen for the bees to survive the winter; bee colonies may only be fed where the survival of the colony is endangered due to climatic conditions. In such case, bee colonies shall be fed with organic honey, organic sugar syrups, or organic sugar.

BEE HEALTH AND WELFARE - for the purposes of protecting frames, hives and combs, in particular from pests, only rodenticides used in traps, and appropriate products and substances authorized for use in organic production shall be permitted. Beehives may be disinfected with physical treatments such as steam or direct flame. The male brood may be destroyed only for the purpose of isolating the infestation of Varroa destructors. In cases of infestation with Varroa destructor formic acid, lactic acid, acetic acid and oxalic acid, as well as menthol, thymol, eucalyptol or camphor, **may be used.**

PROCESSING AND HANDLING - the processing and handling of bee-derived products should also follow organic guidelines. This includes using organic-approved equipment and avoiding the use of synthetic additives in processing.

CERTIFYING BODIES - organic bee products are typically certified by accredited organic certification bodies or agencies. These organizations inspect and verify that the beekeeping and processing operations comply with organic standards.

PROTECTED DESIGNATIONS OF ORIGIN (PDO)

Product names registered as PDO are those that have the strongest links to the place in which they are made.

Products: food, agricultural products and wines.

Specifications: The entire process of production, processing, and preparation needs to happen in that particular area. This implies that for wines, the grapes must only be sourced from the region in which the wine is produced.

Example: The first cross-border Polish product is honey from the Sejny region/Lazdijų/Lazdijų krašto medus; producers from both Poland and Lithuania jointly submitted for registration!

Labeling is required for food and agricultural products, optional for wine.

PROTECTED GEOGRAPHICAL INDICATION (PGI)

PGI highlights the connection between the product's name and its particular geographic location, where a product's reputation, quality, or other attributes can be primarily traced back to its place of origin.

Products: food, agricultural products and wines.

Specifications: The majority of items go through at least one stage of preparation, processing, or manufacture locally.

Example: Polish types of honey protected under this scheme include Drahim honey, Kurpie honey and heather honey from the Lower Silesian Forest.

Labeling is required for food and agricultural products, optional for wine.

TRADITIONAL SPECIALITY GUARANTEED (TSG)

Traditional speciality guaranteed (TSG) is a European quality seal given to goods with historic names that highlight the goods' distinctive qualities. Goods bearing the TSG mark must be produced using conventional raw materials or according to a customary recipe that has been passed down through the generations. A product's name that has been registered as a TSG guards against abuse and misrepresentation.

Products: food and agricultural products.

Examples: Polish items with the TSG mark include półtorak, dwójniak, trójniak, and czwórniak, which are variations of mead**.**

Labeling is required for every product.

QUALITY/SAFETY STANDARDS CERTIFICATION

FDA

IFS

ISO 9001EN - 2015 Quality management systems – Requirements

ISO 2005, Halal i Kosher

NON-GMO CERTIFICATION- STANDARDS

Given that all food products may include traces of genetically modified organisms, the FDA discourages the use of the label "GMO Free." There are GMO labeling thresholds set by the European Union, Australia, and other nations. According to the EU legislation, every food product with a GMO composition of more than 0.9% must list the GMO ingredient on the label (to see the text of the regulation click pls below icon – there is no Turkish translation).

The amount of pollen in honey ranges from about 0.1% to 0.4%. GMO markers may only be found in protein and its average content in pollen is 0,2%. Therefore, any evidence of GMOs in honey will be much below the 0.9% threshold that has been set by nations all around the world to require GMO labeling.

Because the amount of GMOs in honey never goes beyond this limit, honey does not need to be designated or labeled as a non-GMO food. While honey, like most other foods, may not be totally free of genetically modified organisms, it nevertheless meets the criteria for non-GMO food set by the European Union, Australia, and other nations. That non-GMO certificate in the case of honey is only a marketing trick!

OTHER CERTIFICATIONS

Halal certified - the food, cosmetic, and pharmaceutical industries can all benefit from halal certification, which verifies

that a product is made entirely in accordance with Islamic law, contains no “forbidden” ingredients, and has never come into contact with any materials or items deemed “impure.”

Kosher certified - a rabbinic agency’s seal of kosher approval, known as a Kosher Certification, attests to their having examined the product’s ingredients, manufacturing site, and actual production to make sure that no traces of non-kosher materials are present in any of the ingredients, derivatives, equipment, or instruments. Customers are reassured by the Kosher Certified sign that the product itself and its manufacturing process meet all Kosher Law regulations.

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Recent Scientific Advances in Apitherapy Applications and the Effects of Propolis

Prof. Dr. Kemal ÇELİK
Çanakkale Onsekiz Mart University, Çanakkale, Turkey

The Usage of Bee Products in the Treatment of Various Diseases

Sumerians, Egyptians, Indians and Chinese have noted in history thousands of years ago that different foods can be used for the treatment and prevention of certain diseases.In Ayurveda, an ancient Indian health science dating back about 5,000 years, positive effects of food on health were mentioned. The production of functional food (nutraceuticals) against Asian countries which know and use the healing properties of natural herbs and spices has become an important industry especially in the developed countries of the west with the development of innovative technologies.The production of contemporary nutraceutical foods started in Japan in 1980s and continued in America and Europe.Health parameters in humans, and particularly in farm animals, have increased rapidly in the world primarily in the developed countries with regard to the mentality and the demand for healthy nutrition and the increase in the quality of life and productivity.Nutraceuticals, known as

bioactive compounds, can be defined as chemical compounds that are naturally found in animal, vegetable or marine products that can provide the desired health and wellbeing in the living. For example, flavonoids, terpenes, other polyphenolic compounds in propolis, prebiotics in kefir, lycopene in tomato, catechins in tea may be presented as examples of important bioactive components. Today, where the art of modern medicine is desperate, humanity is re-evaluating thousands of years of applications under the light of existing scientific data and applying them with new products. In this context, compounds containing large numbers of bee products have been developed as smart foods, and the developed products, primarily propolis and bee venom, have become widespread. When bee products, such as honey, propolis, beeswax, pollen and royal jelly are investigated, it will be seen that they have been known and used even since ancient times. For example: in ancient China, bee pollen used to be applied as a cosmetic agent that contributed to skin whitening.These products, whose significance have been increasingly recognized via thousands of qualified researches, have started to be used as agents for the treatment of cancer, wounds and burns as well as neurodegenerative, cardiovascular and gastrointestinal system diseases (Yıldız *et al.*, 2013, Baltas *et al.*, 2016).The results of undeniable numbers of scientific researches show that bee venom and royal jelly, primarily propolis, have a potent and powerful source of natural antioxidants that can resist the effects of oxidative stress underlying many known

pathogenesis of disease.In general, these compounds having the phenolic character belonging to the substances expressing the ability to destroy free radicals predominantly determine the antioxidant capacity of bee products (Le Blanc *et al.*, 2009, de Florio *et al.*, 2017).So called these compounds are classified into two main groups: flavonoids and phenolic acids (Rzepecka-Stojko *et al.*, 2015).These natural chemicals are polyphenolic structured vegetable derivatives containing isoflavones and neoflavonoids and various subgroups such as flavonoids, flavones, flavonols, flavanones, flavanonols, flavanols (catechins), anthocyanins, and chachones. The presence of these phenol groups in the molecules of flavonoids, which have very strong antioxidant properties, give them a strong antiradical property and these compounds are effective in stabilizing the resonance that occurs during sweeping free radicals in the body. Phenolic acids are compounds having carboxylic and phenol groups. We can show benzoic acid derivatives and cinnamic acid derivatives to phenolic acids and derivatives in bee products.In general, compounds such as protonated acid, syringic acid, gallic acid, p-coumaric acid have been found in propolis and bee pollen, but caffeic and ferulic acids have been found only in propolis, bee pollen and royal jelly.On the other hand, very strong antioxidant effective artepillin C, chlorogenic acid and 3,5-dicaffeoylquinic acid have been found mostly in propolis.The strong antioxidant activity of these compounds, particularly the prevention of oxidation processes and chelating proxidative metals, has

always attracted attention, thus increasing their possible applications for the protection of human and animal health. To clarify, the number of published studies on propolis alone is 12014, including 8970 in Science Direct, 3025 in pub med, 6 in pub med, and 13 in nature in some journals with very high impact value.In particular, findings from scientific research on propolis have surprisingly increased the interest in this product. When amitins are included in non-phenolic compounds responsible for the antioxidant capacity of propolis, α- and β-amyrins have been reported to show numerous beneficial properties of plant-derived triterpenoid, including antiapoptotic, antioxidant, anti-inflammatory, antifibrotic, and gastro- and hepatoprotective effects (Bonamigo *et al.*, 2017). On the other hand, some propolis studies have shown that β-amyrin is beneficial in the treatment of Parkinson's disease (De Lima *et al.*, 2013, Wei *et al.*, 2017). There is a rational justification for the use of propolis and bee pollen in scientific research, as these products contain bioactive components. However, the application of different solvents, the different structures of the components contained in the bee products, affect the composition of the extracts obtained, and especially the hydrophilic ones are better soluble in polar solvents such as the alcohol group. The properties of the extract depend not only on the nature of the solvent used, but also on the extraction conditions, time and temperature. Most recent research has shown that propolis has more than 500 compounds of polyphenols, terpenoids, steroids, sugars,

amino acids and others, and the science has not yet been able to identify even half of it. The superior antioxidant capacity of propolis depends on these ingredients.

In general, according to literature data, the total phenolic content of propolis extracts ranges from about 30 to 200 mg gallic acid equivalent (GAE) / g dry weight, and the flavonoid content is from about 30 to 70 mg quercetin (especially cardiological drugs, in many immunocompromising products). (QE)/g, and free radical binding activity by potential antioxidant determination (DPPH) ranges from about 20 to 190 grg / ml, (Güneş *et al.*, 2015, Bonamigo *et al.*, 2017). Propolis, which is highly variable according to geographical region differences, botanical origin and regional climatic conditions, contain some phenolic compounds that are different from flavonoids and are believed to be responsible for the antioxidant activity of Brazilian propolis (Zhang *et al.,* 2017). These researchers state that the strong antioxidant activity of Brazilian green propolis stems from 3,4,5-tranphosphinylic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and artepillin C.Most of the studies on the antioxidant properties of propolis have been carried out on cell culture, experimental animals and partly humans.Mujica *et al.*, 2017, evaluated the effects of oral oral administration of propolis solution (twice daily, 15 drops, and 90 days) in Chile and investigated the effects on oxidative status and lipid profile.The results showed that 90-day propolis supplementation resulted in a 67% reduction in thiobarbituric acid reagent content (TBARS;

lipid peroxidation derivative products) and a 175% reduction in reduced glutathione (GSH) levels compared to control group levels.The net changes observed for both parameters were more significant in the propolis-supported group than in the placebo group. Furthermore, on the 90th day of propolis supplementation, an increase in HDL concentration was observed compared to the control group value. Jasprica *et al.*, (2007), in a study they performed, respectively, superoxide dismutase (SOD), glutathione peroxidase (SOD), antioxidant enzymes propolis extract (total daily flavonoid dose 48.75 mg) for 30 days to investigate what changes. Malondialdehyde (MDA), a marker of catalase (CAT) and lipid peroxidation, resulted in a 23.2% reduction in MDA after 15 days of propolis administration. When SOD activity was investigated after 30 days, an increase of 20.9% was detected. Interestingly, however, MDA concentration was found to be similar to the initial value at the end of the application and no effect of propolis application on the parameters studied in female animals was observed (n) 15.Thus, these researchers concluded that the effect of propolis depends on both time and sex, and concluded that only the effect of propolis on lipid peroxidation may exist.The effect of Brazilian green propolis supplementation on antioxidant status in patients with type 2 diabetes mellitus (T2DM) (Zhao *et al.* 2016) was investigated and the 18 weeks of propolis application (900 mg / day) has been found to increase serum GSH and total polyphenol levels and serum carbonyls (protein oxidation markers), as well as to

decrease lactate dehydrogenase activityIn addition, Brazilian green propolis did not affect serum glucose, glycosylated hemoglobin, insulin, aldose reductase and adiponectin. The results showed that propolis affects oxidative stress in type 2 diabetic patients but does not affect diabetes parameters.

Neuroprotective Effects of Propolis

Since vital risks occur in neurodegeneration of mitochondrial damage and oxidative stress, the research findings point out that propolis compounds may contribute to neuroprotective effect due to their antioxidant properties. Brown propolis extracts (WEBP) from two different regions of Iran have been reported to be effective against oxidative damage caused by cerebral ischemia in a mouse stroke model (Bazmandegan *et al.* 2017).Regardless of the geographical source of propolis and the doses used, it was found that WEBP treatment caused significant restoration of antioxidant enzyme activity, decreased lipid peroxidation and infarction volume compared to control group. In addition, the study showed improvement in neurological damage measured by the Bederson scale. Findings obtained in another study conducted by Ni *et al.*, 2017, showed that pre-treatment with Brazilian green propolis reduced H 2 O 2 by investigating SH-SY5Y cells. The findings also suggest that the synapse effect of propolis also increases the expression of critical factors of brain-derived neurotrophic factor (BDNF) and activity-regulated cytoskeleton-related protein (Arc). These results suggest that the authors demonstrate

their protective abilities against neurodegenerative damage associated with cognitive impairment caused by propolis, Alzheimer's disease or aging, with an antioxidant effect. Similar findings display resemblance to those obtained by Nana Ware *et al* .In a study in the same field, the neuroprotective activity of ethanolic extract of Indian propolis (MEEP) in a rat model of Alzheimer's disease was investigated and it was found that propolis caused a decrease in MDA levels in rats due to its high antioxidant properties in important cognitive disorders. In addition, propolis administration has been found to cause dose-dependent acetylcholinesterase inhibition, increase in brain monoamine level, and also increase memory deficits. Findings suggest that more than one mechanism may be involved in the neuroprotective effect of propolis. Jin *et al.*, 2015, reported that pinocembrine, one of the most abundant flavonoids found in propolis, inhibits 6-hydroxydopamine- (6-OHDA-), induced oxidative stress, indicating that it is protective against Parkinson's disease. Researchers have shown that pinocembrine administration inhibits paraquat-induced lipid peroxidation, protein carbonylation, protein nitration and also oxidation of thiol groups in mitochondrial membranes of SH-SY5Y cells. A potent antioxidant, it also activated Nrf2 translocation and increased levels of the glutamate-cysteine ligase regulatory subunit (GCLM), glutamate-cysteine ligase catalytic subunit (GCLC), GSH and HO-1.

The neuroprotective effect of another compound, also abundant in propolis, caffeic acid phenethyl ester (CAPE),

against dopaminergic neuronal loss induced by 6-OHDA in rats has been demonstrated. In this study conducted by Barros Silva *et al.* in 2012, it was observed that hydrogen peroxide production in brain striatum homogenates decreased during treatment with CAPE. CAPE has also been shown to be capable of scavenging ROS by neutralizing unpaired electrons of DPPH, but it has been found that 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl does not affect the affected brain regions. In addition, CAPE inhibited 6-OHDA-induced metal levels (Cu, Fe, Mn and Zn), as well as inhibited mitochondrial permeability transmission (MPT), a neuronal death device that triggered cytochrome C release and caspase-3 activation. The scientists conducting the study concluded that CAPE could be a promising compound in the treatment of Parkinson's and other neurodegenerative diseases based on the findings obtained and the ability to cross the blood-brain barrier. In a similar study, Mahmoud *et al.*, 2017, showed that CAPE, in turn, modulates the JAK / STAT signaling pathway in rats as well as protects the brain against hexavalent chromium toxicity by preventing oxidative / nitrosative stress. Researchers have suggested that the inflammation caused by Cr (VI) in rats together with oxidative stress can also directly activate the JAK / STAT signaling pathway in the cerebrum of these animals, and this is confirmed by phosphorylation of STAT3 mRNA and protein in the cerebrum with JAK2 mRNA and protein expression.The authors state that CAPE reduces the JAK2 / STAT3 signal by alleviating oxidative / nitrosative stress, and this is evidenced

by a significant reduction in protein levels in the CAPE-treated group in JAP2 and STAT3 mRNAs.

Effectiveness of Propolis on Reducing Side Effects of Chemotherapy

Chemotherapy is an aggressive chemical drug therapy used to destroy rapidly growing cancer cells in the body. As cancer cells grow and divide faster than other cells, they usually refer to chemicals used to treat cancer. Numerous studies have shown that propolis can be used as a potential natural antioxidant to alleviate the side effects of chemotherapy. Mitomycin C, cisplatin and doxorubicin are anticancer drugs used in conjunction with radiation or surgery. Unfortunately, their administration to patients also causes various side effects and causes serious injury to various organs and deteriorating living conditions. So-called these harmful effects are also known to cause oxidative damage. Numerous cancer studies that have been conducted for many years have recently shown that propolis is an important and potent apoptotic compound in this context. Current treatments, such as chemotherapy, radiotherapy, and immunotherapy, are based on a method known as apoptosis. In this method, cancer cells are killed by activating proteins called caspases.

In a study, it has been reported that the hydroethanolic extract of Indian propolis (HEIP) has protective effects against mitomycin C- (MMC-), and its partly free radical scavenging and inhibitory effect on lipids causes genotoxicity

and cytotoxicity in cancer cells (Kumari *et al.*, 2017).Potential genotoxic and cytotoxic effects of MMC in the bone marrow were found to cause densities and increase in apoptotic cells in the microcarticle cells compared to the control group individuals, and a decrease in the ratio of monochromatic erythrocytes (NCE) in the polychromatic erythrocytes (PCE) was observed. Chemotherapy is known to have side effects associated with fertility. Again, Kumari *et al.* 2017 investigated the possible effects of Indian propolis (HP) on MMC-induced testicular toxicity. The findings were evaluated by measuring antioxidant / oxidant biomarkers in testis tissue homogenate and it was concluded that even single dose administration of HP was effective in this process while MMC treatment caused long-term oxidative stress. According to the research findings, a significant decrease in MDA level and a slight increase in GSH and CAT activity were observed. MMC administration also led to a decrease in testis function (testicular weight, sperm count, sperm motility and normal head morphology), alleviated by HP administration. Alyane *et al.*, 2008 found that propolis extract significantly reduced peroxidative damage in cardiac mitochondria following injection of doxorubicin in acute doses. Propolis also regulated mitochondrial MDA formation and superoxide anion production as well as respiratory control rate.

Use of Propolis in Cardiovascular Diseases

It has been suggested that the antioxidant properties of propolis can modulate markers of cardiovascular disease.

Salmas *et al.*, 2017, reported that oxidative changes in renal tissue of chronic hypertensive rats could be prevented by propolis, CAPE and pollen administration. According to the findings of these investigators, the total antioxidant status (TAS) and paraoxonase (PON1, an important antioxidant) activity in the renal tissue of hypertensive rats induced by N ω -nitro-L-arginine methyl ester- (L-NAME) was significant. While total oxidant status (TOS), asymmetric dimethylarginine (ADMA, endogen inhibitor of NO synthase) and nuclear factor kappa B (NF- κ) B, regulated by the intracellular redox status were significantly increased. Researchers report that all the degraded parameters are improved by the combined application of propolis, its important compound, CAPE and pollen.

It is stated that pretreatments of Malaysian propolis (MP) improve the negative effects of isoproterenol-induced myocardial infarction in rats. MP is a propolis source which exhibits high total antioxidant activity by both DPPH and FRAP methods. The findings obtained from a study conducted by Ahmed *et al.*, (2017), have shown that isoproterenol administration leads to significantly higher lipid peroxides and reduced activities of cellular antioxidant defense enzymes in the myocardium. In addition, administration caused changes in serum cardiac marker enzymes (creatinine kinase-MB, aspartate transaminase, lactate dehydrogenase and alanine transaminase), in cardiac troponin I levels and in serum lipid profiles. However, administration of MP by ischemic rats was

found to cause the absence of improved histopathological findings in addition to the above biochemical parameters.In a scientific study (Güneş *et al.*, 2017) examining the protective effects of 6 active components of Chinese propolis on oxidative damage to H 2 O 2 -induced cardiomyocytes (H9c2), all compounds tested showed significant cytoprotective activities; however, CAPE showed stronger effects than benzyl caffeate (BZC) and cinnamyl caffeate (CNC), chrysin, pinobanksin, and 3,4-dimethoxycinnamic acid (DMCA). Researchers have published that CAPE, BZC and CHC increase the cellular antioxidant potential of H9c2 (by lowering MDA level and increasing SOD and GPx activities), decreasing intracellular calcium ion level and preventing cell apoptosis. In a similar study, the protective effects of propolis ethanol extract (EEP) were investigated against injury induced by oxidized low density lipoprotein (ox-LDL) in human umbilical vein endothelial cells (HUVEC).Fang *et al.*, (2014), well-known the atherogenic role of ox-LDL in the progression of atherosclerotic cardiovascular disease, EEP pretreatment, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation, ROS and MDA generation, as well as increased antioxidant enzyme activities. -LDL-induced oxidative stress has shown to improve. In addition, EEP reduced ox-LDL uptake by HUVECs and expressed oxene-LDL-upregulated expression of lectin-like oxidized low-density lipoprotein receptor-1 (a critical molecule responsible for ox-LDL uptake by endothelial cells). It has been reported to reduce. They also

state that EEP is important in the protection of dose-dependent EEP against lactate dehydrogenase (LDH) release, caspase-3 activation, and increased apoptosis caused by ox-LDL, as well as a reduction in cell viability. The findings show that EEP protects HUVECs from damage caused by oxy-LDL by partial modulation of LOX-1 mediated oxidative stress.

Tian *et al.* (2015) showed that propolis ethanol extract was able to protect macrophages from ox-LDL-induced apoptosis, and the underlying mechanism was able to partially suppress CD36-mediated oxy-LDL uptake and subsequent activation of the endoplasmic reticulum. On the other hand, another study (El-Awady *et al.*, 2014) reported that propolis may protect against high glucose-induced vascular endothelial dysfunction by reducing oxidative stress in isolated rat aorta. Aortic rings were incubated with propolis extract and high glucose and phenylephrine-induced contraction was induced by disruption of acetylcholine-induced relaxation. On the other hand, researchers have reported a decrease in MDA levels as well as SOD activity and GSH concentration.

Propolis as a preservative in toxicity

The strong antioxidant properties of propolis also support its application as an agent that prevents or alleviates harmful oxidative processes caused by various factors such as trichlorfon, tebuconazole, paracetamol, methylmercury, or UV irradiation. Propolis, trichlorfon-stimulated prooxidant / antioxidant and changes in hematological parameters have

been reported to be effective in carp fish (*Cyprinus carpioda*) (Aksu *et al.*, 2016). In this study, fish were exposed to trichlorfon concentrations, a toxic pesticide commonly used in aquaculture, to eliminate fish culture parasites and propolis was applied at the same time.Application with propolis resulted in alleviation of the negative changes caused by trichlorfon in hematological parameters (red and white blood cell counts, hemoglobin concentration, hematocrit, erythrocyte indices, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration). Aksu *et al.* (2016) investigated the treatment of chrisin (a strong flavonoid in propolis) against paracetamol- (PRC-) induced reproductive toxicity in men. Treatment with PRC resulted in sperm motility, antioxidant enzyme activity (SOD, CAT and GPx) and GSH, as well as the rate of dead sperm in the testicular tissue, abnormal sperm cell velocity, and apoptosis and MDA level. CR was found to mitigate the above effects in a dose-dependent manner with higher dose being more effective. The authors concluded that the possible protective mechanism may be due to the antioxidant activity of CR. In the study, restoration of GSH level was observed in treatment with christin and degenerations in leukocytes and hepatocytes were decreased.

Propolis is said to have accelerating effects on healing of wounds due to antioxidant activity. Cao *et al.* (2017) investigated the protective effects of Chinese propolis ethanol extract (EECP) in mice. Research findings have shown that

the expression of antioxidant-related genes such as EECP, HO-1, GCLM, and GCLC induces the expression of propolis and greatly improves the rate of wound healing. Recent research has shown that ethanol extracts of Chinese propolis (EECP) can reduce intracellular ROS levels not only in H 2 O 2-induced RAW264.7 (macrophage cell) cells, but also in normal RAW264.7 cells. This suggests that propolis can reduce not only pathological but also oxidative stress produced under physiological conditions.

Propolis as Cosmetic Additive

Propolis has also been studied for potential application in the field of cosmetics. Research has shown that propolis can be used as a sunscreen agent and can be used as a component of cosmetics (Gregoris and Stevanato, 2010). In a similar study, Gismondi *et al.* (2014) examined the use of propolis added to sunscreens to prevent the cytotoxic and proradical effects of constituents against damage caused by ultraviolet (UV) radiation. Miller essential oil samples were added to the British lavender flower (Lavandula angustifoli) with a 1% pure and 30% ethanol propolis solution and exposed to UV radiation. UV exposure reduced the antioxidant activity of the essential oil (DPPH, ABTS and FRAP), but propolis supplementation not only inhibited this effect, but also significantly increased this parameter in both exposed and unexposed samples. These promising results were also confirmed by the experiment on high metastatic murine B16-F10 melanoma cells. The addition

of essential oil samples to the culture medium resulted in an increase in cellular GPx, SOD and CAT activity, but much less in UV-exposed. However, as in this case, propolis prevented deterioration of oil properties relative to UV rays.

There have been thousands of publications on the biological and health enhancing properties of propolis over the past 40 years. The different biological and health enhancing effects obtained from these studies are summarized in the table below. As an antibacterial activity, propolis has been reported to be effective against various bacterial strains in the laboratory. Many researchers have examined the antibacterial effect of propolis and its extract against gram-positive (Gr +) and gram-negative (Gr-) bacteria and found that it is broadly effective against gram-positive rod bacteria but has limited effect against gram-negative bacilli. In addition to aerobic bacteria, the antimicrobial effect of propolis ethanol extract against 267 anaerobic bacterial strains was examined and bacterial culture generally showed the highest sensitivity to 1 mg / ml propolis ethanol extract. Propolis extract also increases the effect of existing antibiotics. The effect of antibiotics against Staphylococcus aureus (various strains) and Escherichia coli increases with propolis added to the medium. Propolis ethanol extract has been reported to exhibit high antibacterial activity against gram positive cocci (*Staphylococcus aureus*) but low activity against gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The antibacterial activity of propolis is thought to be due to flavonoids and aromatic acids

and esters in the resin. Galangin, pinosembrin and pinostrobin have been identified as the most effective flavonoids against bacteria. Ferulic and caffeic acid also play a role in the bactericidal effect of propolis. Considering the antiviral activity of this miracle natural product, many researchers have found that propolis extract slows the development of infection on plants (such as cucumber mosaic, tobacco spot, tobacco gangrene), animals (HSV-1, varicella zoster and influenza) and humans (human immunodeficiency-HIV). These findings suggest that propolis has a high potential for use as antiviral drugs. Propolis has a lethal effect against influenza virus (type A) in vitro, while aqueous propolis extract greatly reduces the effect of smallpox virus within 15 minutes. Propolis was found to be effective against various DNA and RNA viruses in the laboratory, including herpes simplex virus (type 1 and 2), adenovirus type 2, blistering inflammation virus and poliovirus (type 2). Propolis and virus research today reached the point that the propolis kills many types of viruses and prevents their proliferation.

Antiviral Activity of Propolis

Many researchers report that propolis extract affects the development of viruses caused by viruses on plants (such as cucumber mosaic, tobacco spot and tobacco gangrene), animals (HSV-1, varicella zoster and influenza) and humans (human immunodeficiency-HIV). These findings suggest that propolis has the potential to be used as an antiviral drug.

Propolis has a lethal effect against influenza virus (type A) in vitro, while aqueous propolis extract greatly reduces the effect of smallpox virus within 15 minutes. Propolis has been reported to be effective in the laboratory against various DNA and RNA viruses, including herpes simplex virus (type 1 and 2), adenovirus type 2, blistering inflammation virus and poliovirus (type 2). Propolis kills viruses and prevents their proliferation. Propolis obtained from different sources and Brazilian green propolis has been found to have significant effects against influenza virus.

Viruses on which propolis are effective:

- Adenovirüses
- Herpes Simplex
- Influenca A and B
- Newcastle
- Polio
- Vaccina
- Rotavirus
- Vesicular stomatitis, Coronar Virus

Anticarcinogenic Function of Propolis (Antitumor / Anticancer Effect)

Many investigators have reported the antitumor effect of propolis in vitro and in vivo. Propolis has been found to inhibit the growth of tumor cells and some compounds responsible for it have been isolated (Alyane *et al.*, 2008). The synergy between propolis and anti-cancer agents is particularly interesting. In

a trial on mice, flavonoids in propolis were found to play a protective role against the toxic effect of chemotherapeutic agents or radiation, and the hope that this protective effect would have similar results in humans is gradually strengthening.When propolis is used together with a combination of antioxidant therapy, it increases the effect of chemotherapy, eliminating side effects on leukocytes, liver and kidneys and allows high doses. Active compounds isolated from Brazilian propolis have been shown to inhibit the growth of liver tumor cells and stop tumor cells in the S phase. A compound obtained from aqueous solutions of propolis (PRF-1) has been found to exhibit antioxidant activity and has cytotoxic effect on human liver cancer cells and human lung cancer cells HLC-2.Two studies showed that propolis-containing local therapy eliminated human papilloma virus (HPV) infection, which can cause uterine cancer, the most common type of cancer in women, within six weeks.Propolis (50 and 150 mg / kg) and some isolated polyphenolic agents (caffeic acid, caffeic acid phenyl ester, and quercetin) reduced the number of tumor nodules in the lung. The use of propolis, caffeic acid and caffeic acid phenyl ester (50 mg / kg) has been reported to be a useful tool to control tumor growth. Although most polyphenols have anti-metastatic effect, caffeic acid phenyl ester from poplar propolis and Artepilin C compound from Baccharis propolis have been identified as the most potent antitumor agents. Regular use of propolis as a food supplement provides a protective effect against cancer-causing mutations in humans. Liang *et al.* (2019) report that CAPE, a propolis

content in the treatment of nasopharyngeal carcinoma, inhibits proliferation and metastasis of cancer cells and may be a potential therapeutic compound in the fight against nasopharyngeal cancers. In another study, Frion Herrera *et al.* (2019) state that Cuban propolis can be used as new chemosensitizing agents against drug-resistant human colon carcinoma cells in the international phytotherapy journal.As it is known, melanoma is a malignant tumor that starts in melanocytes and has the highest mortality rate among all cutaneous tumors. In a study conducted in China, Zheng *et al.* (2017) showed that Chinese propolis (CP) has a strong antitumor effect against various cancers. They demonstrated the combined effects of antiproliferation and anti-inflammation of CP on suppressing the progression of human melanoma cell line A375. These investigators report that inhibition of autophagy in CP-treated cells reduces the antitumor effect and attributed autophagy to CP-induced apoptosis. Kabała-Dzik*et al.*, 2018 found that flavonoids, the bioactive components of propolis, exhibit cytotoxic activity and cause cell cycle arrest and programmatic death of MDA-MB-231 and MCF-7 in breast cancer cells. Japanese researchers Endo *et al* (2018) Brazilian green propolis and a cinnamic acid derivative artepilin C increases the anticancer activity, cancer cells are triggered by propolis is expressed that autophagy triggered death in cancer cells.

Frozza *et al.* (2017) showed that red propolis-enriched fractions enhance apoptotic effects in human cancer cells through mechanisms involving mitochondrial degradation.

Therefore, it has been stated that red propolis fractions contain candidate agents for the treatment of adjuvant cancer and more extensive research will be useful in this field. Ryu *et al.* (2017) observed in a study they conducted that chris, a propolis component, stimulates mitochondrial-mediated apoptosis and ER stress, initiating cell deaths by regulating signaling pathways responsible for proliferation of prostate cancer cells. Similarly, Chinese scientist Ren *et al* in 2016 showed that the combination of galangin and berber, propolis components, in esophageal cancers can provide promising treatment for patients with esophageal carcinoma. A number of similar studies conducted in Taiwan have shown that CAPE can be a potential therapeutic agent for patients with advanced prostate carcinomas in prostate cancer. Again, a group of Thai researchers found that propolis extracts from the northern part of Thailand show pharmacological properties with both antioxidant and anticancer activities and propolis extracts can be considered as an extremely useful agent in the treatment of cancer patients.

Polish researchers Czyżewska *et al.* (2016) have reported that the proapoptotic activity of propolis is very effective on squamous cell carcinoma cells in human tongue.In particular, these investigators examined the applicability of chrysin, galangin, pinocembrine, caffeic acid, p-coumaric acid, ferulic acid, and mixtures thereof, one of the active components of propolis, to the squamous cell carcinoma cell line of human tongue (CAL-27) and its molecular mechanisms.MTT test

results, also known as analysis of metabolic components important in cell growth, showed that a mixture of EEP, polyphenols and polyphenolic compounds in a dose-dependent manner for CEP-27 cells was cytotoxic for cancerous cells. Prangsaengtong *et al.* (2016) reported programmed deaths of cancer cells by inhibiting the process of lymphangiogenesis in an in vitro model of krisin.

Egyptian researchers Motawi *et al.* (2016) in the study carried out through the national cancer institute, tamoxifen (TAM) used in the treatment of breast cancer tamoxifen (TAM) to increase the antitumor effects of propolis and especially an important content of caffeic acid phenyl ester (CAFE) investigated. The results showed that the adjuvant use of CAPE and TAM improved anticancer activity in both in vitro and in vivo models through their apoptotic and angiostatic potentials. Demir *et al*., (2016) in a scientific study conducted in Turkey, investigated antiproliferative and proapoptotic activities of Turkey propolis in human lung cancer cell lines and aimed to show the cytotoxic effects of propolis ethanolic extracts on lung cancer and possible mechanisms. The cytotoxic activity of E5 on A549 cells was described using the MTT assay. The mechanisms involved in the cytotoxic effect of propolis on A549 cells were then examined for apoptosis, cell cycle using mitochondrial membrane potential and flow cytometry, endoplasmic reticulum stress using RT-PCR and caspase activity using lumometric analysis. Among the findings, propolis showed selective toxicity to A549 cells compared to

normal fibroblast cells and A549 cells stopped the cell cycle in the G5 phase, stimulating endoplasmic reticulum stress, caspase activity and apoptosis and reducing mitochondrial membrane potential. Researchers have found that propolis can be used as a therapeutic component in the future as an important component in cancer prevention and treatment.

American researcher Patel S., (2016) stated that propolis is effective against brain, head and neck, skin, breast, liver, pancreas, kidney, bladder, prostate, colon and blood cancers, and added thatpropolis is effective in the inhibition of matrix metalloproteinases, anti-angiogenesis, metastasis, in preventing cell cycle arrest, in inducing apoptosis and in reducing harmful side effects of chemotherapy. Patel stated that this effect was due to caffeic acid phenethyl ester, chrisin, artepiline C, nemorosone, galangine, and cardanol which are antitumor activity components in propolis. A group of researchers from Coomonwealth Medical School, Bordonaro *et al.*, (2014), reported that an effective approach to combination therapy in colorectal cancers (CRC), such as apoptotic inducing drugs (eg, LBH589 and histone deacetylase inhibitors), fermentable fiber, dietary supplements of propolis and coffee extracts can effectively inhibit neoplastic growth in the colon.

In an article by Kasala *et al.* (2015) from India, it was pointed out that propolis is one of the most widely used herbal remedies in Asian countries and they report that it is still one of the the most important products offering health benefitsdue

to its multiple biological activities such as antieustrogenic, antibacterial, antitumoral, antioxidant, anti-inflammatory, anti-allergic and anti-diabetic activities of crisin, which is one of the propolis compounds. The author states that chrisin, whose activity is the highest, is the most promising material with anticarcinogenic properties among its multiple pharmacological effects. These researchers found that chrisin prevented cancer in in vitro and in vivo models in their research and expressed that chris was effective in activities such as stimulation of apoptosis, alteration of cell cycle and inhibition of angiogenesis. Tolba *et al.* (2013) from America reported that propolis increases docetaxel and paclitaxel cytotoxicity with adjuvant effect, especially in prostate cancer treatment. Hsu *et al.* (2013) found that CAPE preferentially inhibits S and G2 / M-phase cell cycle and initiates apoptosis in human cervical cancer. Recent scientific research has shown that CAPE treatment suppresses tumor growth and Akt signaling (three major signaling pathways found to be important in cancer; (PI3K) / AKT kinase chain, protein kinase C family (PKC) and mitogen-activated protein kinase (MAPK) / Ras) in human prostate cancer cells. The combined treatment of CAPE with chemotherapeutic drugs has synergistic suppression effects. Pharmacokinetic studies show that intraperitoneal injection of CAPE at a concentration of 10 mg / kg is non-toxic. CAPE makes cancer cells susceptible to chemotherapy and radiation therapies, causing death. Chen *et al.* (2008) found that propolis and its content, CAPE, is a potent apoptosis-inducing agent in

human pancreatic cancer. Shimizu *et al.*, (2005) from Kobe University, Japan, investigated the effects of artepilin C on colon carcinogenesis in propolis. They state that artepilin C is a bioavailable antioxidant capable of inhibiting the oxidation of intracellular DNA by incorporation into intestinal Caco-2 and hepatic HepG2 (a human liver cancer cell line) cells without any conjugation. The researchers found that artepillin C inhibited the growth of cancerous cells in a dose-dependent manner in the treatment of human colon cancer. Propolis is known to exhibit a wide range of activities, including antibiotic, antiviral, anti-inflammatory, immunostimulatory and tumor carcinostatic properties. Recent research has shown and published propolis-induced apoptosis in human hepatocarcinoma cells (SNU449).

Another researcher, Juanes *et al.* (2019), investigated the effect of red propolis and L-lysine on angiogenesis and tumor growth in a new hamster cheek pouch model inoculated with Walker 256 tumor cells (since the biological behavior of this tumor is similar to what occurs in humans, it is an important experimental model that allows the development of therapies), and when red propolis and L-Lysine were administered after tumor inoculation, they found that they inhibited tumor angiogenesis in the new hamster cheek bag model.Kebsa *et al.* (2018), found that Algeria propolis inhibited the transport function of the pgp-pump(P-glikoprotein (PgP)/multidrug resistant proteins) directly in resistant human lung adenocarcinoma cells, that it stopped G0/

G1cell cycle by increasing DOX accumulation as intracellular anticancer drug and that it reversed multidrug resistance by increasing apoptosis induction. Thus, they concluded that propolis could be developed as a chemotherapeutic agent to reverse multidrug resistance. Chen *et al*, (2003) in a Thermal cycle (TC) / Hyperthermia (HT) and propolis synergy study conducted in 2019, reported that they observed that, in addition to the administration of TC HT, propolis increased the anticancer effect on pancreatic cancer 1 cancer cells through mitochondria-dependent apoptosis pathway and cell cycle arrest.

The results of a study carried out by a group of scientists from the Republic of South Korea are quite interesting, since the consumption of cigarette / tobacco, which is an important cause of cancer cases, leads BaP (the natural carcinogen present in tobacco)one of tobacco carcinogens and nicotine, the addictive stimulant alkaloid, to settle in the body and the excretion of them with the urine could reduce the risk of disease in the presence of propolis.

Propolis and Oral Health

Many studies have shown that propolis is a multi-purpose agent in oral health. Vlachojannis *et al.*, University of Freiburg, Department of Surgical Dentistry and Periodontology (2018) state that alternative treatment options are needed to decrease oral pathogen levels in terms of oral health as well as general health due to the continuous increase in antibiotic resistance.

The researchers evaluated the in vitro antibacterial potential of *Spilanthes oleracea* and propolis extract and found that propolis has potent antimicrobial activities. Propolis mouthwash has been reported to be effective and safe in the treatment of severe oral mucositis, especially in the oral side effects after chemotherapy. Nikajima *et al.* (2016) stated that the application of propolis may be effective in suppressing the metabolic changes caused by periodontopathic bacteria that increase the risk of various systemic diseases. Researchers state that periodontitis is one of the most common causes of tooth loss worldwide and that the prevention of propolis has been increasing recently. Although the botanical origin and geographic location varies, the science today says that propolis has a strong antimicrobial activity against pigmented anaerobic periodontal pathogens. Given the increased resistance to anaerobic bacteria; this effective antimicrobial activity of propolis is promising in the treatment of mouth diseases. Head and neck cancers, affecting approximately 650,000 people annually and causing 350,000 deaths, are in 6th place in cancer-related deaths worldwide. Oral cancers are among the most common head and neck cancers and more than 90% have oral and oropharyngeal squamous cell carcinoma features (OSCC). The overall five-year survival rate of OSCC patients is approximately 63%; this is since therapeutic drugs are not sufficiently effective. Today, it is argued that caffeic phenethyl ester, which is a natural component of propolis, may be an alternative treatment for oral cancer. Recent studies have shown that CAPE treatment

can effectively suppress proliferation, survival and metastasis of oral cancer cells. CAPE treatment inhibits Akt signaling, cell cycle regulatory proteins, NF-κB function, as well as matrix metalloproteinase (MMPs), epidermal growth factor receptor (EGFR), and Cyclooxygenase-2 (COX-2) activity. Therefore, CAPE treatment triggers cell cycle arrest and apoptosis in oral cancer cells. According to evidence showing that abnormalities in EGFR / phosphoinositide 3-kinase (PI3K) / protein kinase B (Akt) signaling, in NF-κB function, in COX-2 activity and in MMP activity are frequently found in oral cancers, that CAPE will be effective in the survival and clinical progress of oral cancer patients of Akt, EGFR and COX-2, as well as in the treatment of advanced oral cancer patients is included in the literature.In a study conducted with red Brazilian propolis, propyl gallate, catechin, epicatechin and formononetin, which are natural components of propolis, were administered after five weeks of tumor stimulation and caused significant reductions in tumor formation (Pinheiro *et al.*, 2014). Santiago *et al.* (2016) from Brazil, which has a very intense consumption and export of propolis, investigated the effects of an odontological product containing propolis in a scientific study and investigated low concentrations of chlorhexidine in human monocytes. Cell marker expression, nuclear factor kappa B (NF-κB) signaling pathway, production of pro- and anti-inflammatory cytokines, and bactericidal activity of these cells against Streptococcus mutans were evaluated. Scientific data have shown that the combination of propolis and chlorhexidine may support the

recognition of antigens by monocytes, activate the NF-B communication pathway, and increase the bactericidal activity of human monocytes against *S. Mutans*.

Antifungal Function of Propolis

Poplar propolis, the bee product with the highest antifungal activity, has been tested against 40 fungi, containing *Candida albicans, Candida glabrata, Candida krusei and Trichosporon spp* strains and it was determined that it has an antifungal effect on *Candida famata, C. glabrata, C. kefyr, C. pelliculosa, C. parapsilosis ve Pichia ohmeri*, which are kinds of fungi that cause spoilage in fruit juices. Propolis extract has been confirmed to have antifungal effect on 17 pathogenic fungi. Some researchers have reported that propolis ethanol extract has an inhibitory effect on 60 yeast strains and 38 fungus strains. Propolis is also used in the treatment of chronic fungal sinusitis patients. As is known, vulvovaginal candidiasis (VVC) is the second most common form of vaginitis. The demand for new treatment alternatives is becoming increasingly important, especially for treatments with less side effects, better tolerability, and lower costs, yet still offers better quality of life for disease prevention. Felix *et al.* (2019) aimed to investigate alternative therapies in a study, including alternative and complementary therapies used by women for adjuvant treatment of vulvovaginitis caused by Candida species, and found that controlled use of propolis would benefit.

Onychomycosis has been addressed in an important study conducted in Brazil, one of the countries where the use of natural medicines is frequently used within the range of traditional and complementary medicine practices. Onychomycosis is a chronic fungal infection caused by dermatophyte fungi, primarily Trichophyton species. Because of the limited drug potential available to treat general fungal infections and the frequent failure of onychomycosis treatment, new therapeutic resources are being explored. In this context, topical treatment with natural products for onychomycosis is encouraged. In a fungal study, patients with onychomycosis received topical propolis extract for 6 months and the results were surprising. According to the findings, propolis penetrated the tissue very effectively and 56.25% of the patients had complete mycological and clinical treatment of onychomycosis. In this study conducted by Veiga *et al.* (2018), propolis was found to be a promising natural compound for the treatment of onychomycosis, with its ability to penetrate diseased tissue without causing any cytotoxicity and good antifungal performance against species such as *Trichophyton spp.*Another researcher Güneş *et al.* (2018) pointed out that caffeic acid phenethyl ester (CAPE), an important component of propolis, has many biological activities, including antibacterial, antiviral, antioxidant, anti-inflammatory and anticancer effects has a significant therapeutic potential against resistant *C. albicans*. Findings from a study evaluating radical scavenging activity, intestinal cell viability, and antifungal activity of Brazilian propolis byproducts (De

Francisco *et al.*, 2018) suggest that propolis byproduct can be used as a new and rich source of bioactive compound (nutraceutical) for different areas such as food or medicine. In another onychomycosis study, Galletti *et al.* (2017) investigated the antibiofilm efficacy of propolisol on fusarium species originating from onychomycosis and determined that propolis extract isolated and reduced biofilms for *Fusarium sppsolani, F. oxysporum* and *F. subglutinans*Tobaldini *et al.* (2016) found that Candida species prevented the formation of biofilms and destroyed mature biofilms and found a significant reduction in *C. tropicalis* and *C.albicans* spread.The authors state that propolis is an inhibitor of Candida virulence factors and can be an innovative source in the fight against candidiasis. In an original study investigating the antifungal effect of propolis against yeasts isolated from blood culture, Mutlu Sarıgüzel *et al.* (2016) showed that propolis had significant antifungal activity compared to fluconazole and itraconazole against yeasts isolated from blood culture. It is possible to support the most up-to-date research that we tried to rank above with hundreds of new research findings. However, the examples presented are sufficient to express the antifungal activity of propolis.

External Use of Propolis (Skin Wounds, Injuries, Burns)

Propolis is used by people for the treatment of surgical diseases, injuries, and burns. Propolis ointment has anesthetic, bactericidal and wound healing properties as well as blood

and lymph system improvement. Some researchers have emphasized that the wound healing effect of propolis depends on the concentration of propolis in the prepared solution. Propolis skin creams have been found to have beneficial effects on healing burn wounds. The more frequent application of propolis increases the antimicrobial effect and wound healing. Propolis is used in skin care products due to its anti-allergic, anti-inflammatory, anti-androgen, anti-lipase, anti-microbial and collagen synthesis effects. Dermatological and cosmetic use of propolis and extract is quite common. The development, characterization, and preclinical studies of propolis-based wound healing glands based on microemulsifying formulation found in biocellulose membranes have shown that propolis-containing glands create a broad spectrum biomembrane potential and accelerate healing (Marquele *et al.*, 2019). This is a very important result in preventing infection of the wound in human and animal injuries and increasing the healing rate. In a study evaluating the efficacy of propolis on fibronectin metabolism in the healing process of burn wounds, Olczyk *et al.* (2013) observed that in the treatment of propolis burn, fibronectin components reduce the release from healing wounds due to damage treated with silver sulfadiazine. It has been found that the release of synthesized fibronectin molecules decreases with respect to the treatment of wounds with propolis, with respect to damage treated with silver sulfadiazine. The results show that propolis changes fibronectin metabolism during the wound healing process, decreases the disruption of fibronectin

biosynthesis and decreases the wound area. In a study conducted in mice, Romana et al. (2018) found that CAPE resulted in closure of dermal reconstruction and pressure ulcers in the inflammatory response and oxidative damages caused by ischemia and reperfusion injury. A similar study was conducted by Italian scientists Martinotti and Ranzato (2015) and it was stated that propolis can be used in the prevention and treatment of many diseases due to its antimicrobial and anti-inflammatory activities. The same researchers predict that, given the availability, low cost and healing power of this regenerative product, the interest in natural products and its healing potential will increase.

Use of Propolis in Oral and Dental Health

There have been many scientific studies on the use of propolis in oral health. Propolis has found application in oral health especially in most of the developed countries. propolis; it can kill different pathogen microbes such as bacteria, fungi and viruses in the mouth, and also successfully protects against different oral and dental diseases such as oral wounds and ulcers, prosthesis, aphthous stomatitis, gingival recession, periodontitis, gingivitis, tooth sensitivity and tooth decay. is applied (8). Due to its complex chemical structure, pharmacological and healing properties, propolis is considered a very powerful natural product produced by bees. The biggest problem in propolis, as in most bee products, is that the content varies and may contain residues depending on flora and production time. Medical use

of propolis is also difficult because of its variable content and standardization problems. Propolis has no established side effects but may cause allergic reactions in some people who are allergic to bee products. In addition, crude propolis must be purified prior to use and the dose of use must be observed. It should be remembered that propolis is not a medicine that cures all diseases. However, because of the properties described above, the possibilities of using propolis for treatment of human and animal diseases should be sought.

Effect	Propolis Type Tested
Antibacterial	All Propolis Types
Antiviral	All Propolis Types
Antifungal	All Propolis Types
AntiparaSİTİC	Poplar Baccharis, Cuba
Antiulcer (Stomach, Skin, Cheek)	Baccharis, India
Antioxidant	All Propolis Types
Radiation Shielding	Baccharis
Liver Protector	All Propolis Types
Antitumor, Antimutagenic	Poplar Baccharis Cuba, Taivan
Antienflamatuar	Poplar Baccharis, Cuba, Egypt
Immune Support	Poplar Baccharis
Relieving muscle contraction in low doses, muscle relaxant in high doses	Poplar Baccharis
Antidiabetic	Poplar Baccharis
Local Anasthetic	Poplar Baccharis
Healing of cartilage, bone tissue, gums and wounds, and being effective in alleviating and disappearing scars	Poplar Baccharis

Table 24. Some propolis types and their effects

SECONDARY EFFECTS	
Antiosteoporoz	Poplar, Egypt
Estrogenic	Poplar
Against Nasal Inflammation	Baccharis
Against Colite	Poplar, Turkey
Antiallergic	Baccharis
Retarding aging and skin aging	Poplar, Algeria
Preservation of different foods	Poplar, Argentina, Egypt,Baccharis

Table 25. Secondary effects of some propolis types

Side Effects of Propolis

The use of propolis for various purposes (in cosmetics, etc.) has caused some allergic events. Propolis and its components, together with isoprenyl caffeate, cause a very strong allergy. No side effects other than allergic activity of propolis have been reported.

biological activity	active substance(s)
Antibacterial	pinosembrin, pinobanksi, isalpinin, galangin, ferülik acid, caffeic acid
Antimicotic	aromatic acid and esters, kaempferol-7,4'-dimetil eter pinobanksin-3-aseat pinosembrin caffeic acid, sakuranetin
Anticandida, Antiseptic	Pinosembrin , benzoik acid
Antiviral	cafeic acid, luteolin, kuersetin, 7-methoxykuersetin, 3.7-dimethoxykuersetin
Antitumor	cafeic acid fenil ester, asasetin, artepillin c, kuersetin krisin

biological activity	active substance(s)
Inhibitor effect	cafeic acid esters, phenolic other compounds
Local anesthetic	pinosembrin, pinostrobin,cafeic acid esters
Capillary strengthening	kuersetin; luteolin'in 3',4'-dimetil eteri
Anti-inflammatory, Antioxidant	cafeic acid, bisabolol, flavonoids
Anti-diabetic	pterostilbene
Healing of gastric ulcer	luteolin, apigenin, pinosembrin, galangin, krisin
Wound healing	phenolic acids, flavonoids

Table 26. Biological activities and active substances of propolis

Check Yourself

1. **How many chemicals does propolis contain?**
   a. only 25
   b. 225
   c. 250
   d. 500

2. **The superior antioxidant capacity of propolis depends on factors such as,**
   a. polyphenols
   b. terpenoids
   c. steroids, sugars, amino acids
   d. all

3. **Propolis, which is highly variable according to:**
   a) geographical region
   b) botanical origin
   c) climatic conditions
   d) all answers are correct

4. Biological functions of propolis are:

a. hemolytic activity
b. anti-inflammatory activity
c. anticancer, antibacterial, antifungal, antiviral activities
d. all answers are correct

5. What are the pytochemicals present in bees propolis that can be indicated for management of Rheumatoid arthritis ?

a. Its true because, prolonged use of conventional anti-rheumatoid is detrimental hence need for alternative therapy
b. Its not true because no alternative treatment for rheumatoid arthritis
c. Rheumatoid arthritis is a spontaneous disease, no intervention is required
d. Rheumatoid arthritis is a disease that affects only men.

6. Generally, the total phenolic content of propolis extracts expressed in gallic acid equivalent (GAE)/g dry weight ranges?

a. from about 3 to 20 mg
b. from about 300 to 2000 mg
c. from about 30 to 200 mg
d. from about 3 to 20 mg

7. The flavonoid content in propolis is:

a. from about 30 to 70 mg quercetin
b. from about 3 to 7 mg quercetin
c. from about 300 to 700 mg quercetin
d. from about to 70 g quercetin

8. Propolis ree radical binding activity by potential antioxidant determination (DPPH) ranges from:

a. about 2 to 19 grg/ml
b. about 20 to 190 grg/ml
c. about 200 to 1900 grg/ml
d. about 2000 to 19000 grg/ml

9. It has been demonstrated that propolis supplementation resulted in:

a. reduction in thiobarbituric acid reagent content (TBARS)
b. reduction in glutathione (GSH)
c. reduction in malondialdehyde (MDA
d. all anwers are correct

10. The propolis is potentially alergic, especially due to presence of:

a. pinosembrin
b. sugars
c. isoprenyl caffeate
d. amino acide.

Answers: 1d, 2a, 3d, 4d, 5d, 6c, 7a, 8b, 9d, 10c

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LEGAL STATUS OF BEE PRODUCTS AND APITHERAPY

Dr. Massimo Canalicchio Dr. Andrea Palomba
Agricoltorı Italianı UMBRIA- ITALY

Introduction

- **Beneficial effects of products obtained by beekeeping.**
- Beyond producing honey and beehive products as royal jelly, bee pollen and propolis, bees are crucial to maintaining healthy ecosystems and securing food supplies. In traditional medicine, people have long used natural bee products due to their biological actions and enhanced health attributes.
- During recent years, **natural bee products have become highly attractive to the pharma and food supplement sector.** Organizations have had the opportunity to look more closely at their pharmacological potentials and therapeutic applications in preventing and coping with diseases.
- **Anti-inflammatory and antimicrobial properties** of natural bee products such as honey and propolis make them helpful in wound healing. They can rapidly **remove bacteria from infected areas**.

Furthermore, their capacity as coating and dressing compounds enhance fibroplasia and angiogenesis in skin damage.

The non-invasive nature that beehive products share provides high quality medical care for children in a safe environment. From immunization to symptom relief, apitherapy can highly strengthen youngster's wellbeing.

- **Bee products like propolis** exert antioxidant, anti-inflammatory and analgesic effects that can improve diabetic complications. By reducing the expression of glucose, propolis acts as an **agent for the treatment of insulin-insensitive diabetes**, while honey offers a healthier alternative to refined sugars.
- The **strong antioxidant and antibacterial properties of propolis** make it one of the best ingredients for balancing problematic skins. Royal jelly also offers nourishing and non-irritating replacements to more aggressive skin-popular products like retinol.
- The **benefits of bee products** offer both treatment and prevention capacities that enhance gastrointestinal health. Experts agree that manuka honey can act as a prebiotic to balance the bad bacteria in the gut; soothing digestion. On the other hand, propolis and bee pollen can induce the growth of healthy bacteria.
- Propolis is widely used in toothpastes and mouthwashes to **decrease permeability and restore**

dentin, counteracting tooth sensitivity. As agents in oral hygiene, manuka honey and propolis effectively fight against oral infections and treat caries or gingivitis amongst others.

- Besides the all-time effective method of bringing relief to sore throats with honey, bee health products have been found to treat **pharyngitis and upper-respiratory tract infections** more effectively than modern medicines.
- Royal jelly's antioxidant properties improve oxidative damage, helping **prevent pancreatitis and other organ inflammations**. Beehive products also offer a protective effect on the liver and pancreas, helping keep the tissues performing at its best.
- Extracts of propolis and pollen are thought to prevent and regulate hypertension by inhibiting the functioning of inflammatory pathways. Cardiovascular disease is one of the most common disorders globally, while bee health products **largely reduce the risk factor** associated with it.
- The **antioxidant nature of bee health products like royal jelly** has proved to prevent chronic diseases and to decelerate the effects of aging. Neuroprotective and nerve-tonic characteristics of honeybee bioactive compounds have the ability to block and treat cognitive behavioral deficits.

Medicines and Food Supplements

How to use bee-based remedies coping correctly with laws

- Research and innovation have played a key role in materializing the benefits of bee products. **From consumable dietary supplements to medicinal remedies, these sectors are constantly working towards exploiting the infinite qualities of bee-based ingredients.**
- Bee products are **rich in salubrious molecules**; such as **proteins**, **simple sugars**, **essential amino acids** and **monounsaturated fatty acids**. Suppliers in the food sector transform bee products into natural and ready-to-use **supplements that can be easily incorporated into human diet.**
- Depending on consumer preferences and needs, food supplements can range from gummies, snacks and sprays to functional foods and honey blends amongst others. **A smart way to integrate an extra boost to the immune system and overall body health with bee products.**
- **Bee health products have been used in traditional healing practices to treat and prevent many types of disorders forever.** Biochemical compounds found in them have been demonstrated to display antibacterial, antiviral, and antiparasitic properties. To offer the best possible treatment to specific

conditions, suppliers in the pharma sector can now offer an array of formats ranging from capsules, syrups, mouthwashes and creams to vials, tablets and emulsions.

- **Food supplements are concentrated sources of nutrients or other substances with a nutritional or physiological effect**. People take supplements **to correct nutritional deficiencies**, ensure that they take in enough of certain nutrients, or **to support specific physiological functions**.
- **Food supplements** are designed to be taken in **small quantities** and are sold in different forms, such as:
- **capsules**
- **powder sachets**
- **drop dispensing bottles**
- Whether you manufacture, sell or import food supplements, you need to ensure that the product complies with **national and EU rules**.
- In the **European Union** specific laws have been approved with reference to **compliance with rules for food supplements, EU Directive on food supplements**
- https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02002L0046-20170726

Nutrition claims

- EU law permits certain nutrition claims, which you may use if:
  - you can prove that your product complies with the official definition
  - the product complies with the conditions for making the nutritional claim (example: 'salt-free' can be used only if the product contains less than 0.005 g of sodium per 100 g).
- **Nutrition claims authorized under EU law are updated and available here:**

https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R1924-20141213#tocId21

Health claims

- A regularly updated list of authorised and non-authorised health claims is available in the EU Register of Nutrition and Health Claims
- Food businesses active in the EU can use the authorised health claims only if they comply with the specific and general requirements. National authorities monitor the use of claims through inspections and legislation.
- To make a claim not already in the EU Register of Nutrition and Health Claims the information you will need to provide is listed under **Article 15** of the EU regulation .

- Further information on the **authorisation procedure** appears on the European Commission's food portal.
- More specifically it can be found the procedure to be followed for health claims on Required information for nutrition and health claim applications and with regard to national authorities taking part in the EU National authorities for submitting nutrition and health-claim applications .
- **Health claim** can be used only if it is displayed together with the following **information on the product's label**, presentation, and advertising:
  - a statement indicating the importance of a balanced diet and healthy lifestyle
  - the **quantity of the food and pattern of consumption required** to obtain the claimed beneficial effect (example: '30 g of walnuts consumed per day will improve the elasticity of blood vessels')
  - where appropriate, a statement addressed to persons who should avoid using the food (example: '**Not suitable for pregnant or breast-feeding women**')
  - a warning for products that are likely to present a health risk if consumed to excess.

Labelling in the European Union

- **Labelling requirements**
- **Food supplements must comply with general food labelling rules and display**:
  - portion of the product recommended for daily consumption
  - warning not to exceed the recommended daily dose
  - statement that food supplements should not be used as a substitute for a balanced diet
  - statement that the product should be stored out of the reach of young children.
- **Warning**: The labelling, presentation or advertising of food supplements **are forbidden from** featuring claims that the product prevents, treats, or cures a disease.

Bee products and their use as ingredients in pharma and food supplements

- **Apitherapy products.**
- In all four countries participating to the project, **Türkiye, Lithuania, Poland and Italy, there are firms producing medicinal beekeeping products**, mostly from **propolis**, **pollen** and **royal jelly**, recommended as associated to conventional medicines. Few companies are producing **bee venom** for the **pharmaceutical** or **cosmetic industry**.

- The use of those **natural ingredients** is regulated by the **Directive 2004/24/EC** on traditional medicine, based on natural pharmaceutical products officially used since at least 30 years, of which at least 15 years in the European Union and with sufficient data proving that they are not dangerous for health and effective vs demonstrated experience and use.
- Also concerned is the European Community Catalogue on **medicines for human use** by **Directive 2001/83/CE**, in which the medicine for humans is **defined as**:
  - any substance or combination of substances presented as **having curative or prophylactic properties against human disease**;
  - any substance or combination of substances that can be used by humans administered to humans for the purpose of **restoring, correcting or modifying physiological functions, exercising an immunological or metabolic pharmacological action**, or to **establish a medical diagnosis** (see picture below).

Regulatory pathway	Main requirements on safety and efficacy	Where to apply
Traditional use registration (Article 16a(1) of Directive 2001/83/EC)	▸ No clinical tests and trials on safety and efficacy are required as long as sufficient safety data and plausible efficacy are demonstrated ▸ Involves assessment of mostly bibliographic safety and efficacy data ▸ Must have been used for at least 30 years, including at least 15 years within the EU ▸ Are intended to be used without the supervision of a medical practitioner and are not administered by injection	▸ National competent authority of a Member State for national, mutual recognition and decentralised procedures
Well-established use marketing authorisation (Article 10a of Directive 2001/83/EC)	▸ Scientific literature establishing that the active substances of the medicinal products have been in well-established medicinal use within the EU for at least ten years, with recognised efficacy and an acceptable level of safety ▸ Involves assessment of mostly bibliographic safety and efficacy data	▸ National competent authority of a Member State for national, mutual recognition and decentralised procedures ▸ EMA if centralised procedure applies
Stand-alone or mixed application (Article 8(3) of Directive 2001/83/EC)	▸ Safety and efficacy data from the company's own development or a combination of own studies and bibliographic data	▸ National competent authority of a Member State for national, mutual recognition and decentralised procedures ▸ EMA if centralised procedure applies

Table 27. Substances that can be used by humans administered to humans for some purposes

Apitherapy products.

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natural pharmaceutical products officially used since at least 30 years, of which at least 15 years in the European Union and with sufficient data proving that they are not dangerous for health and effective vs demonstrated experience and use.

- Based on the **Directive 2004/24/EC** has been created a community list of natural substances with use in the medicinal field for a sufficiently long period to be considered harmless under normal conditions of use
- **Community monographs** have been released relating to traditional medicines that contain the scientific opinion of the Committee based on the evaluation of available scientific data (well estabilished use) or on the historical use of the product in the European Community.
- **130 monographs are currently available on the website of EMA (European Medicine Agency)** with publication of summary of recommendations in a clear and simple language for the public.
- **Use of medicinal beekeeping products in veterinary medicine.**
- The use of medicinal beekeeping in veterinary medicine is at initial stage as there is a lack of scientific studies, but perspectives are very promising, so scientific research projects in collaboration with universities, laboratories and other public institutions are foreseen be developed.
- The main reason is that beehive products have as potential sources **many flower species**, and therefore they have

extremely variable characteristics, and it is necessary to determine the overall quality of each product and/or its therapeutic properties to create a quality mark and a certification of this products.

- In the treatment of **skin lesions** with honey it is possible to combine propolis or phytotherapy principles (e.g. essential oils) to deter animal's licking, to repel flies and to enhance the healing effect.
- Veterinarians recommend organizing **practical training courses to form specialized veterinarians** on this issue and creating a working group of veterinarians specialized in apitherapy, to share skills and updates on the theme.
- **Doses and application protocols** should be moreover defined for their topical and oral use in veterinary for the different animal species. **A datasheet model for the description of the clinical cases** and the collection of national experiences should be implemented to create a database.
- About the **venom therapy in veterinary medicine**, starting from the animal welfare practices, it should start with the determination of whether the patient is allergic by administering a small amount of venom intradermally. If there are no adverse reactions, then it is possible to increase gradually over several weeks until the maintenance dose is achieved.
- The **bee wax**, deriving from the glandular secretion of bees, is for the most part reused in the same production

cycle beekeeping, to produce wax sheets.

- However, **bee wax is also used in numerous fields**, mostly in sectors others than medicine, i.e. as a waterproofing and protective material, in the precision engineering industry, for paints and for some products of the house, for wood and leather processing, in art, in medicine, in some pharmaceutical preparations, cosmetic and candle making industries.
- **Use of natural compounds from beekeeping in the food sector.**
- Even larger than in medicine, for humans or for animals, is the **use of natural compounds from beekeeping in the food sector**, that has been established under several European Regulations:
    - EC Regulation 178/2002: Foods
    - Directive 2002/46/EC: Food supplements
    - EU Regulation 2015/2283: New foods
    - EC Regulation 1924/2006: nutrition and health claims (claims) proposed on food labels and/or advertising
    - EC Regulation 1170/2009: lists of vitamins and minerals and their forms that can be added to foods, including dietary supplements
    - EC Regulation 353/2008: implementation rules for applications authorizing the health claims provided for in Article 15
    - EC Regulation 1169/2009: amending regulation 353/2008

- EC Regulation 116/2010: amending Regulation (EC) No. 1924/2006 as regards the list of nutrition claims
- EU Regulation 1169/2011: food labeling
- EU Regulation 432/2012: list of authorized health claims,
- Regulation 609/2013: infant formula, for special medical purposes, whole food ration
- EU Regulation 907/2013: rules relating to questions concerning the use of generic descriptors, names traditionally used to indicate the peculiarity of a category of food or drink, produced with at least 20 years of use within the European Union.
- EU Regulation 828/2014: information absence or reduced presence of gluten.

Borderline food/medicine bee products and their classification

- With regard to the authorization process, consumer information and distribution channels, European and national legislation consider food supplements (FS) in the same way as "food". **One of the more complex issues regarding FS is the fact that many substances are used both as ingredients of FS and as active ingredients of medicines**. At the moment, there are no unambiguous scientific and regulatory criteria to distinguish the food use from the pharmaceutical use of a substance and the two fields of application frequently overlap.

- The European Commission has tried to put order for **"borderline" products**, identifying the following criteria to define a FS:
  - a product intended for the general population that is healthy or has a risk factor for the development of disease;
  - a product whose consumption favors the maintenance of a physiological function of the body or the reduction of a risk factor;
  - a product that cannot boast preventive and therapeutic effects against a pathological condition;
  - a product characterized by nutritional and health indications (claim) proposed on the labels and/or with advertising in accordance with the current Community regulation on the matter.
- Another element which, for some molecules, is used to distinguish the use of a certain active ingredient as a supplement or as a medicine is the dose. When the molecule is offered in doses that overlap the **recommended daily intake** (**RDI**), it is classified as FS; **if the proposed consumption unit significantly exceeds the RDI, the preparation should be classified as a medicine**.
- If a product, even if already used as a food supplement, were to be offered in a "therapeutic" context, it would therefore fall into the category of medicines.
- An important aspect refers to those products defined as **"medical devices"** which could probably contain the

substances mentioned above. In particular, some substance-based medical devices could represent a sort of "middle ground" between food supplements and medicines.

- For **beekeeping substances** also, such as **propolis**, it could be considered the use as medical device in case of treatment targeted to stop inflammation of the throat or an incipient cold. The difference with respect to the FS lies in the fact that medical devices may contain substances intended to be used in humans for the purpose of diagnosis, prevention, control, therapy or mitigation of a disease through a main action not exerted by pharmacological or immunological, nor by metabolic process, but whose action can be assisted by such means (**EU Regulation 2017/745**) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017R0745 .

Conclusions

- The **European Parliament resolution** of 1 March 2018 on prospects and challenges for the EU apiculture sector (2017/2115(INI)) had **stressed the strategic importance of beekeeping for pollination and therefore for a sound agriculture within a natural context**.
- **Beekeeping products other than honey are increasingly sought after on the market** for their use in formulations of **healthy food supplements** or in **pharmaceutical preparations** as **medical devices** dedicated to human or animal care.

- In recent years, **traditional medicine of oriental origin**, which has used products such as pollen, propolis and royal jelly for decades, has tended to move closer to **conventional Western medicine** and to **extend the use of products derived from beekeeping to preventive medicine**.
- Some countries including **Poland**, **Lithuania** and **Türkiye** have acted as a bridge for this rapprochement, while **Italy** has only in the last decade shown sensitivity towards the importance of using such products especially as food supplements.
- The **European Commission**, together with the **European Parliament** and the **Council**, and the **EFSA** (European Food Security Agency) have followed this evolution over the years, considering the scientific results achieved in the use of these products in the pharmaceutical sector as positive and also in terms of **economic advantages for beekeepers**, **thus promoting their survival and future development**.
- The foundations have therefore been laid for **legislation** that is **able to incorporate in a clear and updated manner the positioning of the different products and their distinction between food supplements and medical devices and the role of apitherapy**.

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Bee Products for Traditional and Complementary
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SONÇAĞ YAYINCILIK MATBAACILIK
İstanbul Cad. İstanbul Çarşısı No.: 48/48
İskitler 06070 ANKARA
T: (312) 341 36 67
soncagyayincilik@gmail.com
www.soncagyayincilik.com.tr