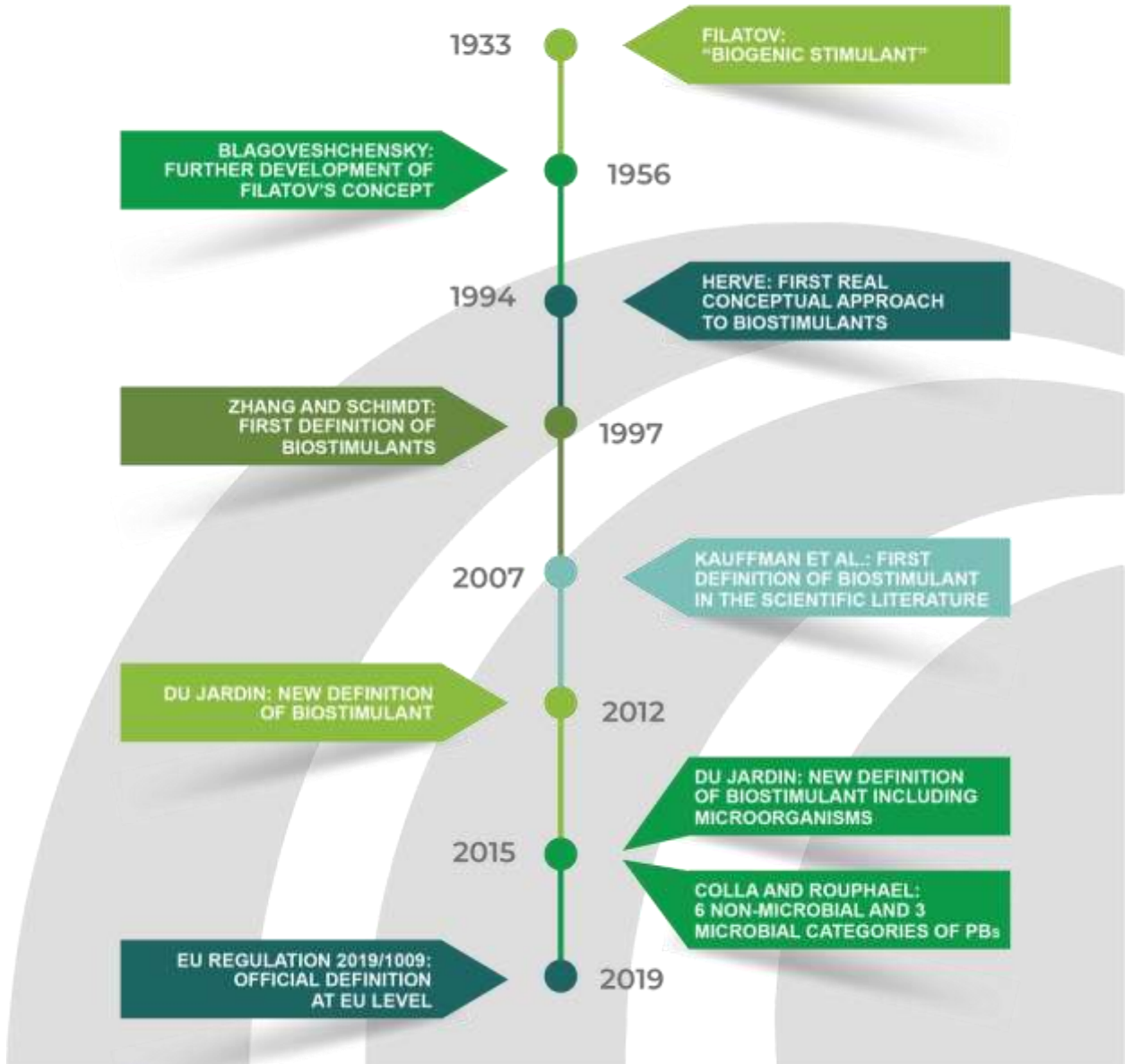
Immunomodulators and biostimulants of natural origin.

Biogenic stimulants accelerate vital processes in the body, increase metabolism, increase the body's resistance to diseases.

The composition of biogenic stimulants consists of a complex set of substances, which has not been sufficiently studied until now.

The first discussion of “biogenic stimulant” theory can be attributed to Prof. V.P. Filatov and was started in 1933 in the USSR. For the first time, he noticed that when the cornea of the corpses was kept at a temperature of 2-4°C and surgically transferred to the eyes of patients, the organ stored in such conditions was better assimilated. According to Filatov, if the tissues isolated from the animal and plant organs fall into unfavorable conditions for living (cold, dark, etc.), biochemical changes occur in them, and in these difficult conditions, they produce some substances that ensure the survival of the living organism. These substances P. Filatov called biogenic stimulants. It is derived from the words "vios" - khaet, "genesis" - birth, emergence, "stimulo" - awakening.

ORIGIN AND MAIN STAGES OF THE EVOLUTION OF THE TERM BIOSTIMULANT



Cold and high pressure (under water) are unfavorable conditions for plants. The work of Gnedkov (1983) is important. He isolated biosed, lekosed, flavosed, sedoglucid drugs from 12 types of succulent plants, which are used in the treatment of inflammation and cancer. According to him, monosaccharides are variable and participate in the formation of organic acids in the dark, and in their decomposition in the light.

Bicarbonic acids, unsaturated aromatic and oxyacids, macromolecular aromatic acids, which are part of the fat group, have been isolated from the preparations containing biogenic stimulants. From its physico-chemical properties, it is known to be resistant to high temperatures, soluble in water, and partially soluble in water.

Filatov proposed that biological materials derived from various organisms, including plants, that have been exposed to stressors could affect metabolic and energetic processes in humans, animals, and plants. further developed these ideas with specific reference to their application for plants, considering biogenic stimulantsas “organic acids with stimulating effects due to theirbasic properties which can enhance the enzymatic activity in plants.

”Filatov’s concept, was, however, not limited to these compounds al one. Herve’s pioneering review provides the first real conceptual approach to biostimulants. Herve suggests the development of novel “bio-rational products” should proceed on the basis of a systemic approach founded in chemical synthesis, biochemistry, and biotechnology as applied to real plant physiological, agricultural, and ecological constraints. He suggests these products should function at low doses, be ecologically benign and have reproducible benefits in agricultural plant cultivation. Zhang and Schmidt emphasized the need for comprehensive and empirical analysis of these products with particular emphasis on hormonal and antioxidant systems as the basis for many important benefits of biostimulants. They discuss the concept of biostimulants as “pre-stress conditioners,” their effects being manifested in improved photosynthetic efficiency, reduction of spread and intensity of some diseases and in better yields. Basak initiated the systematic discussion on biostimulants and created the conceptual preconditions for the formation of present biostimulant science while Du Jardin provided the first in-depth analysis of plant biostimulant science with an emphasis on biostimulant systematization and categorization on the basis of biochemical and physiological function and mode of action and origin. Du Jardin’s analysis and categorization was influential in informing the development of subsequent legislation and regulation in the European Union.

Different animal and plant tissues under the same conditions can produce and accumulate the same substances. The chemistry of biogenic stimulants has not been fully elucidated. Apparently, they represent a complex of substances from which acids of aromatic series, unsaturated dicarboxylic acids, unsaturated aromatic compounds (cinnamic and oxycinnamic acids, coumarin) have been isolated. Biogenic stimulants have some common physical and chemical properties: they are water-soluble, partially distillable with water vapor, heat-resistant (they can withstand sterilization at 120°C).

Preparations containing biogenic stimulants are classified as follows:

1. ***Preparations from plants***

(Extractum Aloes, Extrastum herbae melitoli, Biosedum, Inecosedum, Flavosedum, Sedoglucidum).

2. ***Preparations obtained from microorganisms and plants belonging to the lower class*** (Peloidinum, Peloidodestillatum, Fibs, Gumisolum, Torfogum).

3. ***Extracts from animal organs*** (Sorrus Vitreum, Apilacum, Luronitum, Shonsuridum, Spleninum, Plasmolum, Chole conservata medicata, Haemotagenum liquidum).

4. Those obtained ***from microorganisms*** (moggos) (Pyrogenalum).

Biogenic stimulants drugs

Liquid aloe extract — (***Extractum A1oes fluidum***). It is obtained from the leaves of aloe tree (A1oe arborescens) grown in Central Asia and Transcaucasia. A two-year-old aloe leaf is stored in a dark place at a temperature of 4-8°C for 10-12 days. Then remove the yellow parts and grind it in a meat grinder. 3 times the amount of water is poured into the resulting porridge-like mass and left for 2 hours. Then the mixture is boiled for 2-3 minutes, filtered and the level of oxidation is checked with 0.01 N potassium permanganate. It should be 1500-600 mg O2/l. Depending on the test result, it is diluted with water until there is 1500 mg of oxygen in one liter of liquid. Then 7 g of sodium chloride is added to each liter of solution, the solution is boiled for two minutes and filtered. Clear, extract is poured into vials of 200 ml (for drinking) and ampoules of 2 ml (for injection). Filled ampoules are sterilized at 120°C for 1 hour. The finished product is a clear, light-yellow to yellowish-red liquid with a pH of 5.0-5.6. Stored in a cool, dark place.

It is used in the treatment of various diseases, inflammation of the stomach and duodenum, bronchial asthma and other diseases.

I. M. Kurilenko explains the bioactivity of aloe extract by the fact that it contains lemon, apple, grape, amber and oxyacids. These substances are formed when aluminum is exposed to unfavorable conditions (darkness, low temperature). It was determined that the extract contains Sa, Va, Si, Mp, Ni, Fe and other such trace elements. Organometallic salts together with other bioactive substances determine the effect of the drug.

I. M. Kurilenko et al. proposed to dry biostimulated aloe leaf by lyophilization method. It has been confirmed that the composition and effectiveness of the drug obtained from the leaf collected by the Filatov method is the same as that obtained from the dry raw herb. The composition of these preparations is proven by pop-exchange chromatography, paper chromatography, and complex ionometric titration of the total amount of cations.

The extract obtained from the raw materials dried by the lyofill method is stable and does not form a precipitate for 8 months.

<u>Biosed</u> (*Viosedum*). It is an aqueous extract of freshly harvested large sedum (Sedum maximum) biostimulated topsoil. It is a pale yellow, clear liquid with a characteristic odor, pH 5.0-6.0. It contains 12 mg % of polyphenols based on rutin. It is produced in ampoules from 1 ml.

It is used in ophthalmology, in the treatment of internal diseases, in surgery and dentistry, in the restoration of tissues.

Peloidin (*Peloidinium*). This drug is an extract extracted from the mud with water, and contains complex salts in addition to stimulants (sodium, potassium, calcium and magnesium chlorides, sulfates, iodides, phosphates, carbonates, bromides). 72 l of water, 6.68 kg of sodium chloride are added to 280 kg of mud and mixed, and left at 200C for 3-6 days, then the separation is poured, filtered through the last ultrafilters. The solution purified from microorganisms is heated at 60-700C for 1-1.5 hours and poured into glass bottles of 0.5 l under aseptic conditions.

Peloidin is a clear liquid and should be stored in a cool, dark place.

The density of the product is 1.008-1.01; Ni 7.4-7.8; chlorides 1.15-1.35%; dry residue should be 1.2-1.6%. It is used in the treatment of bacillary dysentery, scurvy, various inflammations.

Peloidodestillate (*Peloidodestillatum*). Medicinal mud is extracted from the harbor mud with the help of steam. Contains volatile biogenic stimulants. 750 ml of product is obtained from 1 kg of mud. 8 g of sodium chloride is added to each liter of the finished distillate. It is produced in ampoules of 1 ml. The drug is a clear colorless liquid with a pH of 6-8.0. Stored in a cool, dark place.

It is used in the treatment of chronic joint inflammation and various eye diseases.

<u>Apilak</u> (*Arilacum*). It is a dry royal jelly obtained from worker bees. It is used for hypotrophy in young children, hypotonia in adults, and it is used as a 0.6% ointment for skin and facial itching (seborrhea).

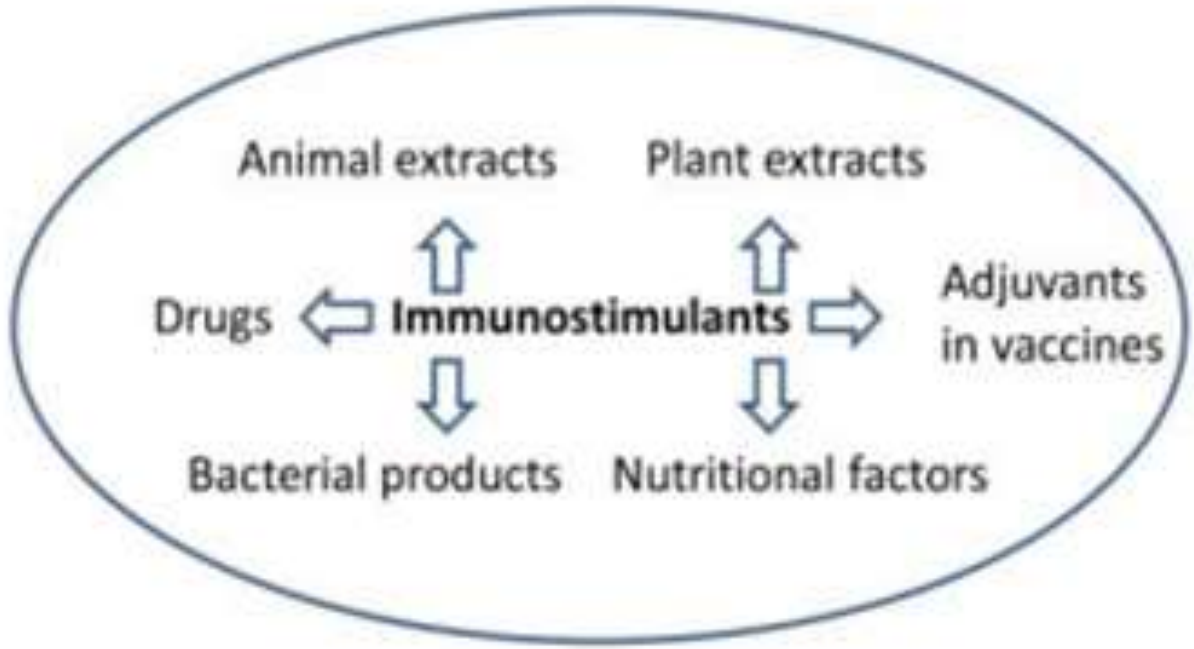
Apilak powder (0.07 g of apilak - 0.93 g of milk sugar), ointment and 0.02 g tablet (under the tongue) are available.

Immunostimulants

Immunomodulators are natural or synthetic materials that regulate the immune system and induce innate and adaptive defense mechanisms. These substances are classified into two types, *immunostimulants* and *immunosuppressants*.

Immunostimulants can enhance body's resistance against various infections through increasing the basal levels of immune response.

There are several types of stimulants with different mechanisms and functions such as bacterial products, complex carbohydrates (e.g., glucans, schizophyllan, scleroglucan, lentinan, statolon, bestatin, acemannan), vaccines, immunoenhancing drugs (e.g., Levamisole, Isoprinosine, Fluoro-quindone, Avridine, Polyribonucleotides), nutritional factors (e.g., vitamins, carotenoids, lipids, trace elements, selenium), animal extracts (e.g., chitosan from shrimp), cytokines (e.g., macrophage activating factor, interferon, interleukin-2, tumor necrosis factor), and plant extracts (e.g., Lectins, mitogens such as phytohemagglutinin, concanavalin A).


Animal extracts
Plant extracts
Drugs
Immunostimulants
Adjuvants in vaccines
Bacterial products
Nutritional factors

Plant-derived immunostimulants. Natural plant product promote various activities such as anti-stress, growth promotion, appetite stimulation, immunostimulation, aphrodisiac and antimicrobial properties, due to the active substances such as alkaloids, flavanoids pigments, phenolics, terpenoids, steroids, and essential oils. Medicinal plants have been known as immunostimulants, growth promoters, immune enhancers, where they act as antibacterial and antiviral agents to the host immune system. Unfortunately, the mechanisms were not understood.

Some medicinal plants were described as following:

a) *Ocimum sanctum* (Tulsi): Leaves of *O. sanctum* containing water-soluble phenolic compounds and various other constituents may act as an immunostimulant. Leaves extract of *O. sanctum* affected both specific and nonspecific immune responses. It stimulated both antibody response and neutrophil activity.

b) *Solanum trilobatum* (Purple Fruited Pea Eggplant): The herbal extract of *S. trilobatum* possesses a broad spectrum of antibiotic, antibacterial and anticancer activities. A study showed that the water-soluble fraction of S. trilobatum significantly enhanced the production of reactive oxygen and decreased the percentage of mortality following a challenge with *Aeromonas hydrophila*.

c) *Zingiber officinale* (Ginger): The extracts of *Z. officinale* contain polyphenol compounds which have a high antioxidant activity. Moreover, it showed a significant increase in proliferation of neutrophils, macrophages, and lymphocytes, as well as it enhanced phagocytic, respiratory burst, lysozyme, bactericidal and antiprotease activities.

d) *Echinacea* (purple coneflowers) and *Allium sativum* (garlic): *Echinacea* and *A. sativum* improved the gain in body weight, survival rate and resistance against challenge infection of *Aeromonas hydrophila*. Both compounds developed resistance to cold stress during the winter season.

Immune stimulation is usually measured using parameters such as an increase in numbers of circulating immune cells, or enhanced phagocytosis after inoculation with a pathogen. It is notoriously difficult to substantiate claims for the prevention of disease, since very large clinical studies are needed for statistical validity, and these are difficult and expensive to perform. However, echinacea is taken widely and the use of an Oriental medicinal plant, astragalus, is increasing in the West for the same indications.

Echinacea, *Echinacea pallida* (Nutt.) Nutt., *E. purpurea* (L.) Moench and *E. angustifolia* DC
(*Echinaceae herba*, *radix*)

Family *Asteraceae*

Echinacea purpurea is an herbaceous perennial up to 120 cm tall by 25 cm wide at maturity. Depending on the climate, it blooms throughout summer into autumn. Its cone-shaped flowering heads are usually, but not always, purple in the wild. Its individual flowers (florets) within the flower head are hermaphroditic, having both male and female organs in each flower. It is pollinated by butterflies and bees.

The alternate leaves, borne by a petiole from 0 to 17 cm, are oval to lanceolate, 5-30 x 5-12 cm; the margin is tightened to toothed.

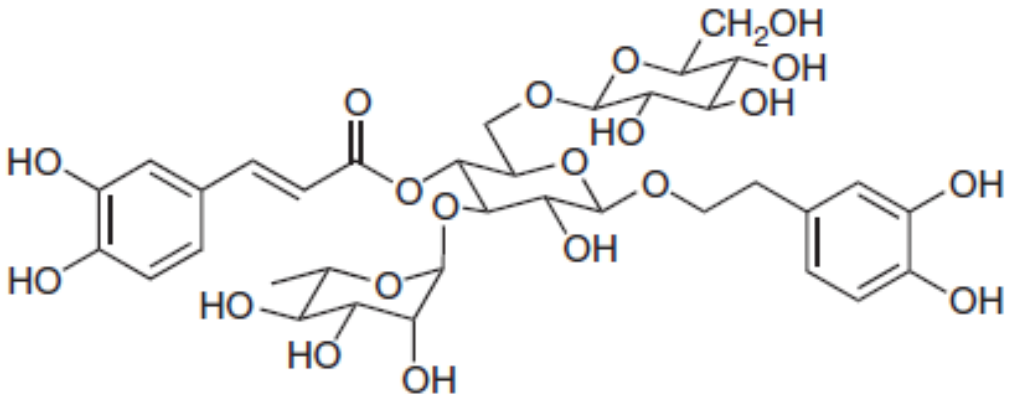
The inflorescence is a capitulum, 7 to 15 cm in diameter, formed by a prominent domed central protuberance consisting of multiple small yellow florets. These are surrounded by a ring of pink or purple ligulate florets. The tubular florets are hermaphrodite while the ligular florets are sterile. The involucral bracts are linear to lanceolate. The plant prefers well-drained soils in full sun. The fruit is an achene, sought after by birds.

Flowerhead

Close-up showing many individual flowers comprise the flowerhead

Members of the genus *Echinacea* (*Asteraceae*) are widely distributed in North America and have a long tradition of use, both by the American Indians and the settlers, who developed the first commercial reparations during the 19th century. Both aerial parts and secondary roots are used. The indigenous people used *E. pallida* in particular for a variety of illnesses, such as pain, inflammatory skin conditions and toothache. The three botanical species are used as immunostimulants in the preparation of phytomedicines to ‘prevent colds and other respiratory infections’. The complex situation regarding species, quality of products made from them and method of production makes an assessment of the clinical efficacy very difficult. *Echinacea* is often combined with garlic, for the treatment of colds and allergic rhinitis.

Constituents. Numerous compounds have been identified, but the most pharmacologically relevant ones are not known. All species contain similar types of compounds, although not necessarily the same individual ones. The most important are the caffeic acid derivatives, including echinacoside (*E. pallida root*), cichoric acid (*E. purpurea aerial parts*) and others, and the alkylamides (found throughout the plant in all three species), which are a complex mixture of unsaturated fatty acid derivatives. Some have a diene or diyne structure (with two unsaturated and two triple unsaturated groups) or a tetraene structure (with four unsaturated groups) linked via an esteramide to a (2)-methylpropane or (2)-methylbutane residue.



Echinacoside

Therapeutic uses and available evidence. Echinacea preparations are available both as traditional herbal medicinal products used to relieve the symptoms of the common cold and influenza-type infections, and as preparations with a well-established use. There is some evidence in the treatment and prevention of respiratory infections, but more limited evidence for slow-healing wounds using topical applications. Clinical evidence for use as an immunostimulant is available for some of the chemically characterized extracts. Overall a series of meta-analyses showed that Echinacea preparations seem to be efficacious both therapeutically (reducing symptoms and duration) and in terms of prophylaxis against the common cold.

A large (755 healthy subjects) randomized, doubleblind, placebo-controlled trial assessed the safety and efficacy of specific *Echinacea purpurea* extract products over a period of 4 months. Participants were given the herbal preparation (standardized to contain 5 mg/100 g of dodecatetraenoic acid isobutylamide; 0.9 ml 3 times a day corresponding to 2400 mg of extract) for illness prevention or placebo. The study concluded that this herbal preparation was safe and, compared to placebo, effective. Consequently the authors state that it can be recommended as a prophylactic treatment. However, *Echinacea* preparations tested in clinical trials differ greatly. There is better evidence that preparations based on the aerial parts of *E. purpurea* might be effective for the early treatment of colds in adults but the results are not fully consistent. A mechanism of action has been postulated by Chicca et al, suggesting that the alkylamides dodeca-2 *E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (A1) and dodeca-2*E*, 4*E*-dienoic acid isobutylamide (A2) bind to the cannabinoid-2-(CB2) receptor and are the main anti-inflammatory and immune-modulatory principles, acting in synergy.

In addition, alkylamides potently inhibit LPS-induced inflammation in human whole blood and exert modulatory effects on cytokine expression, but these effects are not exclusively related to CB2 binding. Echinacea appears to be safe, although allergic reactions have been reported. The risk of interactions seems to be very limited.