

WHITE PAPER

NATURAL SOLUTIONS FROM THE MEDITERRANEAN TO BOOST IMMUNITY

Scientific publications
review



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ABOUT PHARMACTIVE BIOTECH PRODUCTS, S.L.



Pharmactive Biotech Products, S.L. is a privately-owned Spanish company that develops and manufactures **differentiated natural ingredients supported by scientific evidence**. Pharmactive makes these innovative ingredients available to other companies in the Nutraceutical, Pharmaceutical and Veterinary Industries for marketing/distribution. Our aim is **to generate a positive and significant impact on people's health and well-being**.



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MOST COMMON DISEASES OF HUMAN BEINGS



“Common cold” and “flu” are usually caused by different viruses, receiving the name of “acute upper respiratory tract viral infections” [URTIs], which are the most common diseases of human beings. They are mainly caused by single-stranded RNA viruses from the Picornavirus family, known as rhinoviruses. However, there are other possible types of viruses [around 200 identified] involved in the development of this illness, such as coronaviruses, parainfluenza viruses, respiratory syncytial viruses, adenoviruses and enteroviruses. The prevalence of each one can vary significantly depending on the region and year.

“According to the World Health Organization, between 5 and 10% of adults worldwide become infected by influenza annually¹. Approximately 11.5% of all the lower respiratory tract infections were attributed to influenza (54.5 million cases globally)³.”

“Common cold” and “flu” are not only caused by different viruses, but by bacteria and mycoplasmas that provoke similar symptoms and are frequently confused with one another due to the individual variability [table 1]^{4,5}.

In adults, influenza is responsible for the seasonal epidemic outbreaks, meanwhile the rest of viruses usually behave as sporadic or starring in different peaks during the year. Influenza is a virus that spreads easily in human population with periodical epidemic outbreaks^{6,7}. In children of ≤ 5 years old, it must be also considered the respiratory syncytial virus as a seasonal epidemic.





	Viral origin		Non-viral origin	
	Common cold ⁴	Flu (Influenza) ⁴	Mycoplasma ⁵	Bacterial ⁵
Symptoms	Upper respiratory symptoms: nasal congestion and discharge	Lower respiratory symptomatology: cough and fever	Lower respiratory symptomatology: Dry cough and/or clear sputum	Lower respiratory symptomatology: Productive cough and/or purulent sputum
Frequency ⁶	All year (peaks)	Seasonal epidemic (high peak)	5-year cycle	Variable (peaks all year or unknown)

Table 1: summary of URTIs according to the pathogen that causes it.

TRADITIONAL MEDICINAL USE OF OLIVE LEAVES



The olive tree [*Olea europaea* L.] is a perennial tree which originated in minor Asia but has been cultivated throughout the Mediterranean basin for centuries. It has been a source of ancestral remedies and traditionally used as a medicinal plant with different indications in various preparations derived from its leaves, fruits, seeds, wood, bark and oil. It has been part of the traditional human and veterinary pharmacopeia in Mediterranean countries⁸.





Olive is the first botanical species mentioned in the Bible: *"The fruit will be for food and the leaf for medicine"* [Ezekiel 47:12]. The use of olive leaves for medicinal purposes dates back to ancient Egypt, where they were considered a divine symbol as well as a popular remedy to combat fever. Similarly, Greek culture also used it to lower fever.

In 1843 [England], pharmacist **Daniel Hanbury** published a preparation of olive leaves that was responsible for the cure of malaria and associated fevers. Later, in 1898, a preparation of these leaves was recommended by the King's American Dispensatory as an adjunct for regulating fever.

Today, the olive leaf is attracting attention for its myriad functional benefits for the immune and cardiovascular systems.

Trusted origin of raw material

Spain is the world leader in olive production worldwide. Thus, taking advantage of this ancestral know-how about the olive tree, **Pharmactive** relies on a trusted local source to ensure the high quality and pureness of the final extract.

"Immune-boosting botanicals with roots in ancient or Traditional Medicine are factors that strongly resonate with consumers, who perceive them as natural and effective and respect their long history of safe use⁹".



BIOACTIVE COMPOUNDS OF OLIVE LEAVES



In general, the pharmacological properties of olive leaves have been attributed to their phenolic content, and to oleuropein as their main phenolic compound. Oleuropein is formed by esterification of hydroxytyrosol and elenolic acid [a glycosylated monoterpene]. It has been reported in several *in vitro* studies that this compound presents different properties, including antioxidant, anti-inflammatory, anti-atherogenic, anti-cancer, antimicrobial, and antiviral. Additionally, it has been claimed that oleuropein exhibits potent antiviral activities against herpes mononucleosis, hepatitis virus, rotavirus, bovine rhinovirus, canine parvovirus, and feline leukemia virus. Moreover, *in vitro* studies have also shown that oleuropein exhibits a significant antiviral activity against Respiratory Syncytial Virus [VRS] and para-influenza type 3 virus¹⁰.

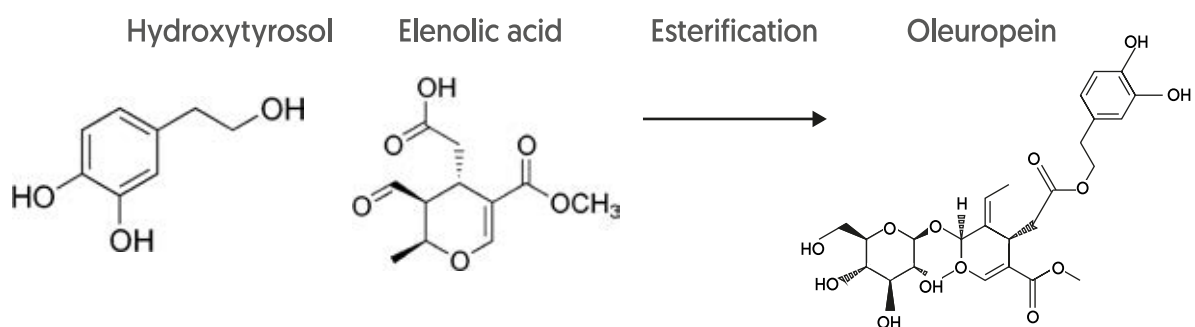


Image 1: Oleuropein formation from hydroxytyrosol and elenolic acid.





NATURAL SOLUTIONS FROM PHARMACTIVE TO SUPPORT IMMUNITY



Pharmactive's new ingredient developments to boost immunity are based on ancient medicinal botanical spices that will build consumer's trust, as natural, effective, and safe solutions.

Olivactive®: support to strengthen the immune system

Olivactive® is a unique olive leaf extract with double standardization: >20% oleuropein and >2% bioflavonoids by HPLC. It is a natural immune booster with microbicidal and immunomodulatory effect^{11,12}.

Bioactive compounds

Olivactive® is standardized to >20% oleuropein and to >2% bioflavonoids by HPLC.

Oleuropein is one of the most characteristic biomolecules from *Olea europaea* L. leaves, which exhibits immunomodulatory¹⁰⁻¹⁴, microbicidal¹⁰⁻¹⁸, and anti-inflammatory¹⁹⁻²¹ properties.

Bioflavonoids are potent antioxidants that have been shown to play a role glycemia cases balance, helping to regulate high levels of glucose in the blood, which is related to a higher risk of infection²²⁻²⁴. It is considered to confer better stability of the extract due to its antioxidant properties and can be used as an identifying profile of the olive to avoid adulteration.





Locally sourced and high-quality standards

Olivactive® is locally sourced from olive trees grown in fields in the Mediterranean and uses an extraction process that is fully controlled and completely traceable, leveraging ancestral know-how in order to provide a final extract of the highest quality and purity.



Main mechanism of action

Olivactive® and its bioactive compounds have shown four main mechanisms of action:

1. Immunomodulation.

The oleuropein present in Olivactive® regulates the immune response of macrophages¹⁰⁻¹³ and granulocytes¹⁴ by controlling:

- The release of Nitric Oxide [NO] and Reactive Oxygen Species [ROS] that exert an **antimicrobial effect**^{10,11}.
- The expression of inflammatory mediators that **promote adaptative immune response** [such as TNF- α , IL-6, or COX-2]^{12,14}.



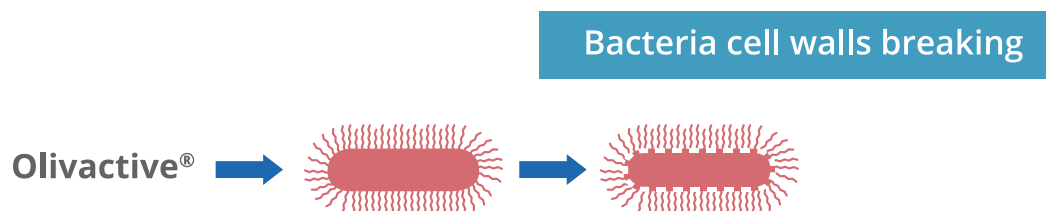


Note. Granulocytes: a category of white blood cells; TNF- α : Tumor necrosis factor-alpha; IL: Interleukin; COX-2: Ciclooxygenase-1.

2. Anti-microbial activity.

The oleuropein present in Olivactive® has shown **strong antimicrobial activity** against **bacteria** [Gram-negative and Gram-positive], **mycoplasma**, and **viruses**^{15,25}. Moreover, it seems to be especially useful in **antibiotic-resistant infections**¹⁸.

The oleuropein breaks down into hydroxytyrosol, which **promotes the breakage of bacteria cell walls**, and damages cellular membranes [via the action of ortho-diphenols]¹⁵.

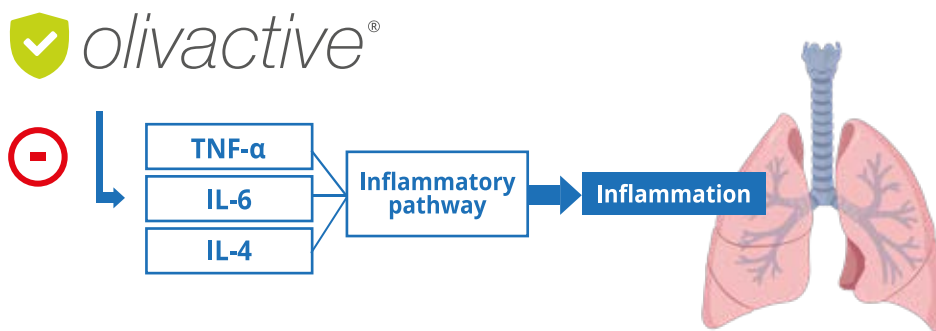




3. Anti-Inflammatory at systemic level.

Bioflavonoids and oleuropein present in **Olivactive®** attenuate the **overall inflammatory state**, reducing inflammation in several cell types¹⁹, including **muscle**²⁰ and **lung**²¹ cells.

Bioflavonoids **regulate the expression of inflammatory mediators**, such as **TNF- α** , **IL-6**²⁰ and **IL-4**²¹.



Note. TNF- α : Tumor necrosis factor-alpha. IL: Interleukin

4. Glycemia balance.

The bioflavonoids present in **Olivactive®** inhibit two enzymes involved in **glucose absorption in the gastrointestinal tract** (α -amylase and α -glucosidase)²⁶ leading to a positive effect on **immune system strength**^{23,24}.

The oleuropein present in **Olivactive®** has shown to **regulate glycemia** through NOX2 [NADPH Oxidase₂] gene expression modulation [which is overexpressed in these cases], contributing to a reduction in the **generation of metabolic oxidative stress**²⁷.

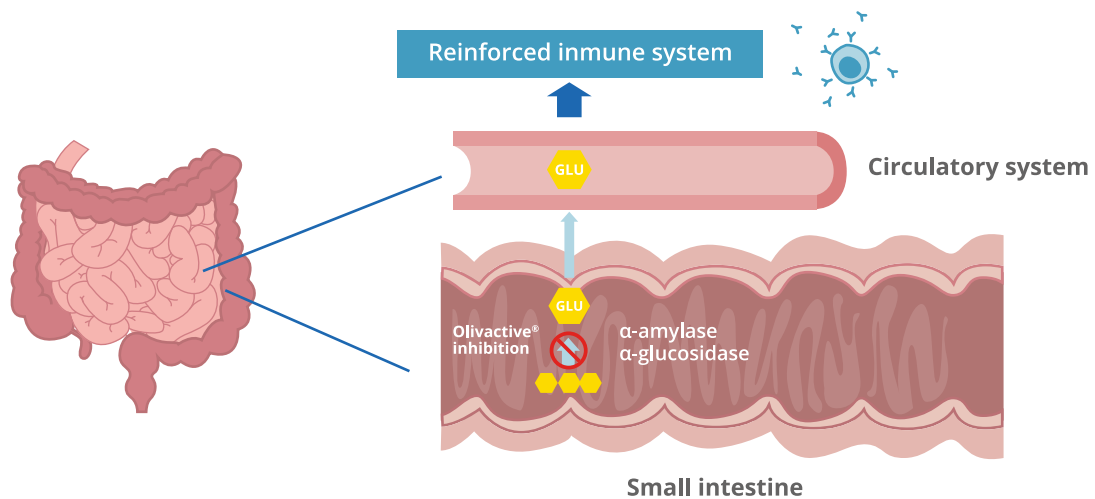




Numerous scientific publications have related **pathological glycemia** [characterized by high levels of glucose in the blood] **with increased frequency of infections and decreased ability to fight them.**

Some of the consequences of unbalanced glucose levels are:

- Defective leucocyte performance.
- Reduced capacity for leucocytes to phagocytize and effectively kill bacteria.
- Less ability for Immunoglobulin G to recognize bacteria²⁸.





Isenolic®: an extra boost to help with viral infections

Isenolic® was born as a sustainable ingredient for prevention and relief of viral infections²⁹⁻³¹. It has a multi-target mechanism of action to stop the virus life cycle, being proved as a natural source of neuraminidase inhibitors, which are recommended by WHO for viral infection management³². It is standardized to $\geq 4\%$ elenolic acid by high performance liquid chromatography (HPLC), which has been reported to exert antimicrobial activity [virus and bacteria]¹⁸⁻³³⁻³⁴.

Scientific studies, since the beginning of the 19th century, have shown the positive effect of elenolic acid in immunity. Accordingly, Isenolic® may help to^{29-31,36-37}.

- Reduce flu and flu-like symptoms.
- Reduce the duration of cold.
- Fight against seasonal flu acting as an additional tool.
- Provide antioxidants, promoting a boost to immunity.

Its activity was tested against the gold standard Tamiflu drug and reached outstanding influenza inhibition values, *in vitro*^{29,30}. Isenolic® treatment inhibited a 65% of influenza A virus activity after the infection (it was close to the 88% exhibited by the gold standard antiviral drug Tamiflu) (Figure 1).

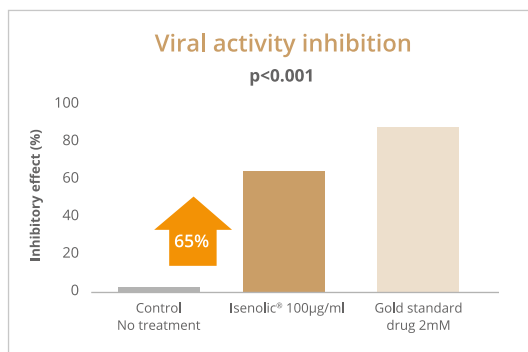


Figure 1: Neuraminidase activity fluorometric assay. Influenza virus used: influenza A [H3N2].



Isenolic® treatment preserved 73% of viable cells after influenza A infection, *in vitro*. This has been attributed to the likely protective effect of **Isenolic®** from cytopathic effect [structural changes] caused by influenza^{29,30}. **Isenolic®** was close to the 93% exhibited by the gold standard antiviral drug Tamiflu [Figure 2].

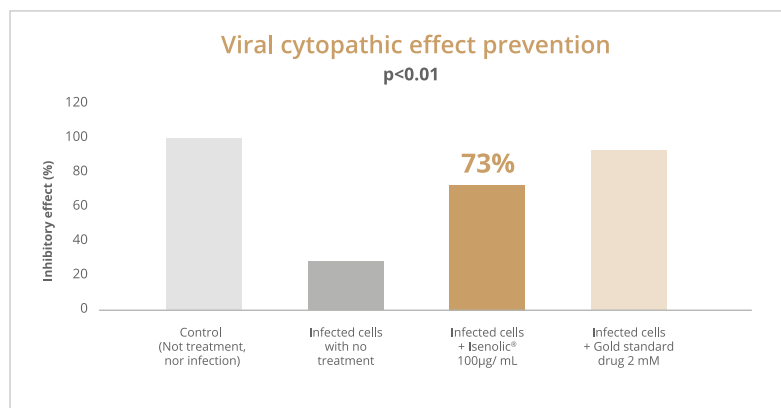


Figure 2: MTT tetrazolium reduction assay. Influenza virus used: influenza A [H3N2].

Multi-target mechanism of action

Isenolic® directly penetrates into the cells being able to act prior and during the infection period thanks to its proposed multi-target mechanism of action:

- 1 Blocks the virus' entry into the cell^{31, 34-37}.
- 2 Stops its replication: neutralizes reverse transcriptase and protease production and reduces essential amino acids production^{31, 34-36}.
- 3 Avoids its liberation: proven neuraminidase activity inhibition³⁷.
- 4 Promotes its destruction: stimulates phagocytosis of the virus^{31, 34-36}.



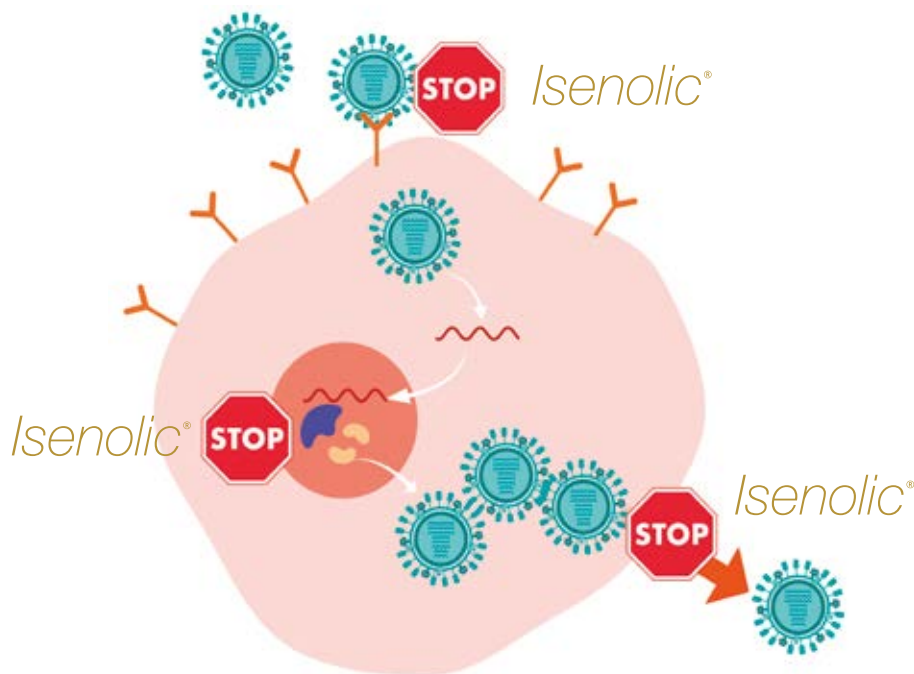


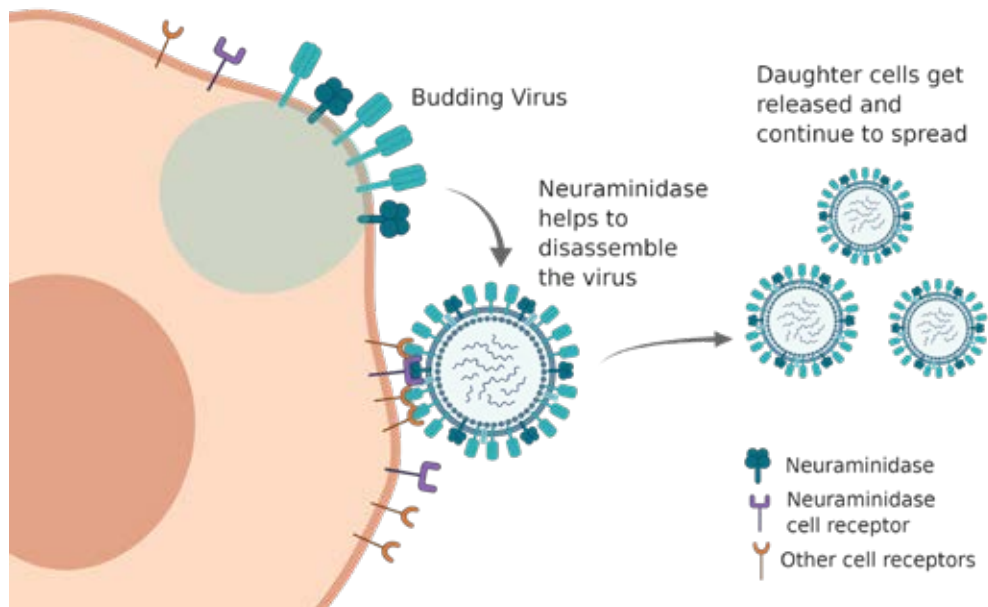
Image 2: Proposed Isenolic® mechanism of action.

Neuraminidase as a key for anti-viral activity

Influenza and other viruses promoting “common cold” and “flu” symptoms follow a special life cycle called lysogenic, which mainly consists of cell infection, entry of the viral genome into the nucleus, replication of new viruses and release. One of the key molecules to complete the cycle is neuraminidase: an enzyme present in the flu virus capsid, that acts to disassemble the virus from the host cell membrane, promoting its release to infect new cells.



Without neuraminidase inhibitor, virus replication proceeds



With neuraminidase inhibitor, virus replication stops

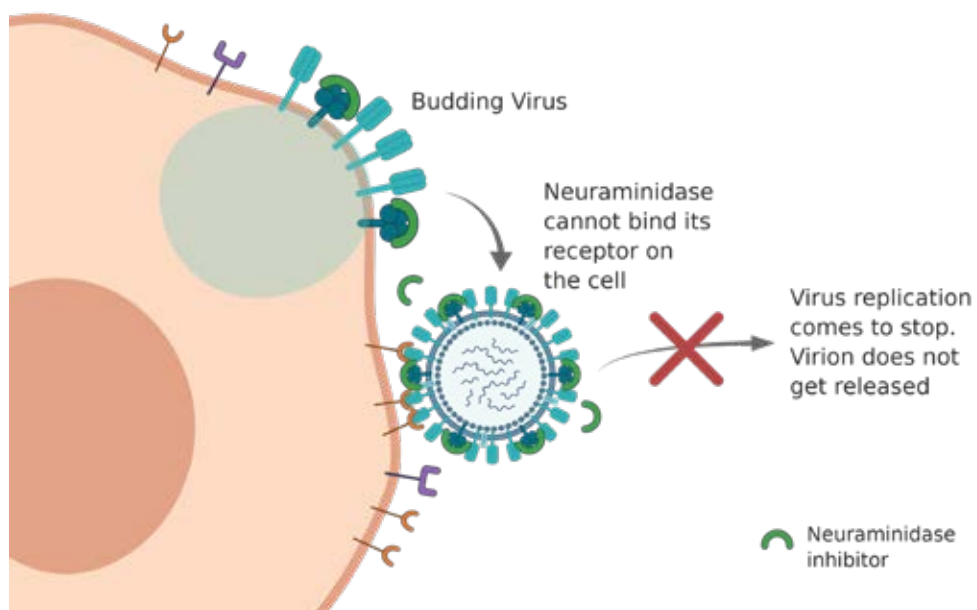
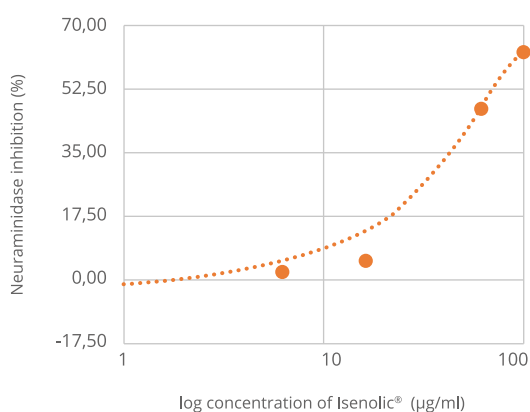


Image 3: Neuraminidase activity explained.





Isenolic® avoids virus release by inhibiting neuraminidase, showing an IC_{50} [concentration of **Isenolic®** necessary to reduce the neuraminidase activity of the virus by half] of 171.5 $\mu\text{g/mL}$, in a cell line especially vulnerable to influenza [MDCK-SIAT1]³⁷.



Concentration of Isenolic® necessary to reduce the neuraminidase activity by 50% (IC_{50})

Figure 3: Viral neuraminidase inhibition assay in MDCK-SIAT1 cells after treatment with **Isenolic®** containing 4 % elenolic acid and infection with Influenza A [H3N2].

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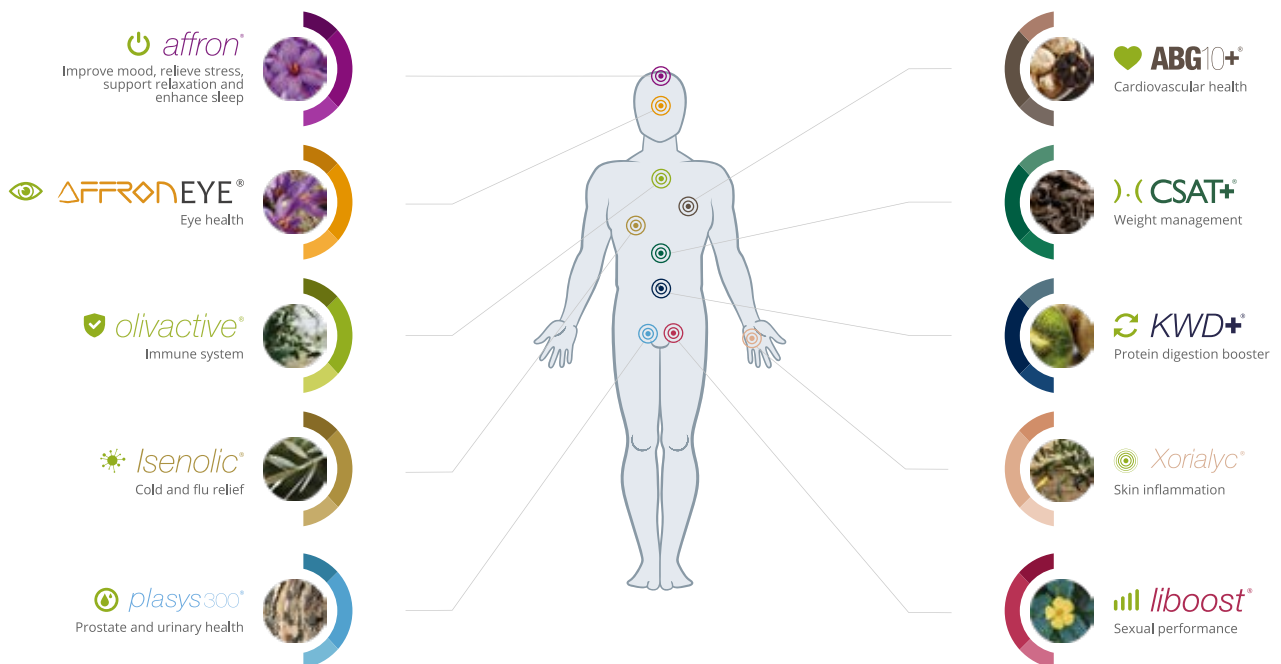


OTHER PREMIUM INGREDIENTS TO WATCH



Pharmactive Biotech Products can offer you a wide range of innovative and patented ingredients from natural origins and backed by our own scientific studies and with a characterized mechanism of action.

NATURAL INGREDIENTS ENDORSED BY SCIENCE



Feel free to contact us
to further information
or to discuss your
business ideas

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