ENOTprost®

FOR MALE WELLNESS

The new ingredient for prostate food supplements

Epilobium angustifolium L. dry extract

standardized to contain 15% Oenothein B

1- Botanical information

Epilobium angustifolium L. (*Onagraceae*), Fireweed or Willow herb, is a perennial herb, growing to 2m at a fast rate, with erect, simple stems and widespread rhizome-like roots. Leaves are alternate, lanceolate and almost stalkless; flowers are clustered in showy, terminal spikes; the corolla is made of 4 petals, pink to purple in color. Fruits are linear pods, which split lengthwise to release seeds; the main feature of the seeds is that they are covered by a whitish cotton-like hair tuft, enabling them to be dispersed by the wind and to travel long distances (anemophylous dispersion). Blooming time is from July to September; the fruits ripen from August to October. The plant is native to the sub-mountainous regions of Europe and Western Asia, where it grows spontaneously in rocky grounds, waste areas and woodland edges (1). The plant is now found all over the world in temperate areas, including Alaska, Tasmania and New Zealand (2). *Epilobium angustifolium* L. is often one of the first plants to start growing after a fire (1), hence the common name "Fireweed".

2- Parts used:

Aerial parts ("herba"), collected in eastern Europe at spring time and then dried. The botanical species is certified by DNA barcoding analysis.

3- Active constituents:

Fireweed contains three major polyphenolic families: **flavonoids**, **phenolic acids** and **ellagitannins** (2,4-6). Flavonoids include both flavonol aglycones (myricetin, quercetin, kaempferol) and flavonoid glycosides (**hyperoside**, isoquercetin, quercitrin and miquelianin) (2,5-6). Miquelianin (myricitin-3-O-glucorinide) is the major flavonoid glycoside from *E. angustifolium* (6) and has been detected only in this species (5), as it is also shown in the ENOTprost metabolic profile at page 4. Phenolic acids include chlorogenic, gallic, cinnamic, caffeic, ferulic acids (2,4-5), while ellagitannins are mainly represented by the macrocyclic ellagitannin **oenothein B** (2,4-5); other minor components are some sterols and triterpenes (2-4,5).

4- Galenic forms:

No monograph on *Epilobium angustifolium* L. is available in the Eur. Ph. 9th Ed.; the dry powdered extract is obtained by **hydroalcoholic extraction** and standardized to contain **≥15% of Oenothein B** (HPLC method (8) validated in EPO Srl R&D laboratory). The extraction method was optimized through **DOE** (Design of Experiment or experimental design) which is based on a statistical approach by doing many factorial experiments, ensuring result validity, reliability, and replicability with appropriate levels of statistical power and sensitivity.

5- Traditional uses:

In Europe the genus *Epilobium* is known as a medicinal plant from the 16th century for the treatment of wounds, to stop bleeding and for the treatment of female disorders (5); an infusion from the leaves was used in folk medicine as an astringent and soothing agent in many **gastrointestinal disorders**, from diarrhea to irritable bowel syndrome (3); the herbal tea was reported as a treatment for migraine headaches, insomnia, anemia, *delirium tremens*, infections and colds (6); it has also been traditionally used as an **antiprostatic agent** in the treatment of benign prostatic hyperplasia (BPH) (2-5) and micturition disorders (2,5).

Topically the plant has been used as skin conditioning, soothing, cleansing and healing agent to treat minor burns, skin rashes and other dermatological complaints (1,3), also in children (3).

Although the mechanism of action of *E. angustifolium* L. is not fully understood, it is has been proposed that **oenothein B** is partially responsible for the *Epilobium* extract bioactivities. Oenothein B has immunomodulatory, antioxidant and anti-inflammatory properties; the combined enhancement of innate immune defenses and protection of host tissues through antioxidant effects could allow oenothein B to optimally provide health benefits. It has been pointed that additional compounds besides oenothein B may contribute to the antitumor properties of the plant. Unfortunately, clear data about the bioavailability of ingested oenothein B are still missing (6,8).

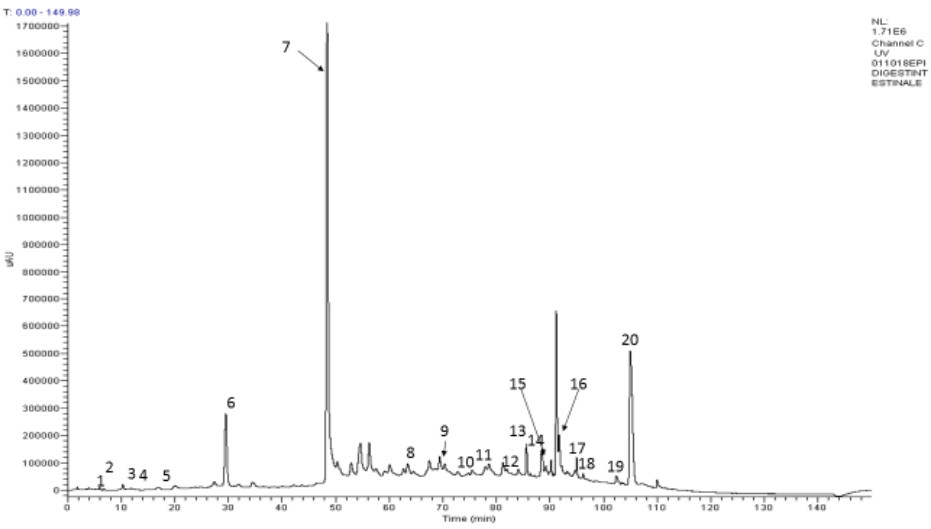
Anti-proliferative and anti-inflammatory effects of Epilobium extracts have been reported *in vivo* and *in vitro* in many studies (5); nevertheless, clinical studies are still lacking.

Thanks to the collaboration with the University of Pavia, EPO Srl has carried out a preliminary study to assess 1) bioaccessibility and 2) bioactivity (anti-inflammatory and antioxidant).

1. bioaccessibility

It is defined as the quantity of a/more compound/s that is/are released from the matrix (in this case from the powdered dry extract) in the gastrointestinal tract, becoming available for absorption (e.g. enters the blood stream) (9). The **metabolic profile** of ENOTprost was studied after oral-gastro-duodenal digestion and duodenal digestion, simulating, in this latter case, the gastro-resistant oral administration.

RESULTS: The oro-gastro-duodenal digestion reduces notably the amount of bioactive molecules, including oenothein B. The duodenal digestion instead still maintains the phytocomplex variety and chemical profile as shown in the chromatogram. In particular the digested extract was rich in 20 compounds among which there are organic acids, hydroxycinnamic acids, many flavonols and the most represented one which is the ellagitannin Oenothein B. Miquelianin is the major flavonoid glycoside that characterizes the *E. angustifolium* species (5) (peak 19).

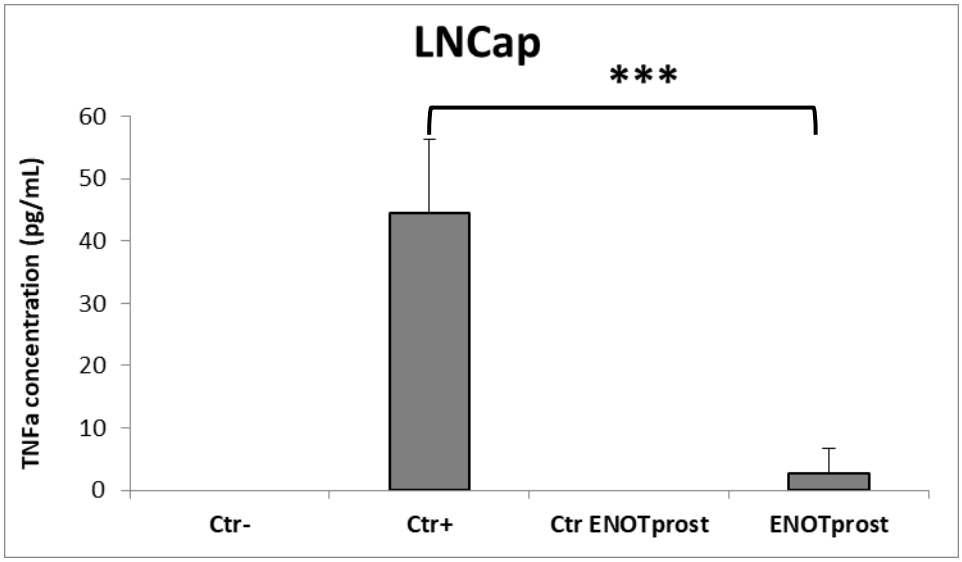


Chromatogram of ENOTprost after duodenal digestion simulated *in vitro* and registered at 250 nm with a RP-HPLC-DAD-ESI-MSn.

2a. anti-inflammatory activity

The anti-inflammatory effect was tested using the LPS-induced inflammation model. Monocyte cells (THP-1 cell line) were inflamed by LPS treatment and induced to produce pro-inflammatory cytokines. In parallel, to test the anti-inflammatory activity of ENOTprost, prostatatic LNCaP cells were pre-treated with ENOTprost and then incubated with the cytokines previously produced by THP-1 cells simulating the inflammation process. Using ELISA test, tumor necrosis factor-α (TNF-α) was measured in LNCaP cells. This molecule is a key cytokine that influences inflammation response and metabolism.

RESULTS: It was evident that the pretreatment with ENOTprost strongly reduces TNF-alpha compared to control cells as showed in the following histogram.



Ctrl^{-}: not inflamed and not treated with ENOTprost

Ctrl^{+}: inflamed but not treated with ENOTprost

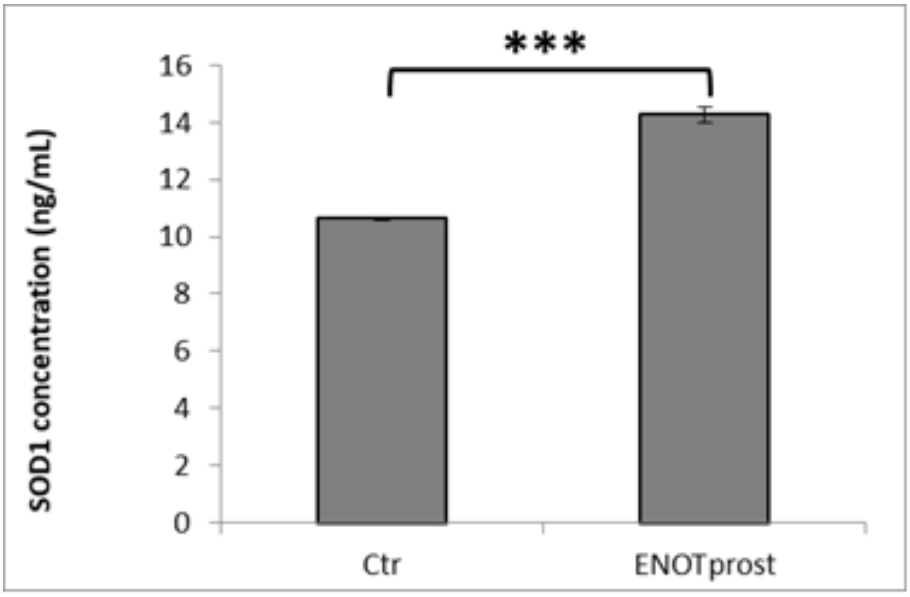
Ctrl^{ENOTprost}: not inflamed but treated with ENOTprost

ENOTprost: inflamed and treated with ENOTprost

2b. anti-oxidant activity

The antioxidant activity was tested studying the cellular antioxidant capacity through SOD1 analysis after ENOTprost pre-treatment. This enzyme catalyzes the dismutation (or partitioning) of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) which is less toxic than O_2 and will be eliminated by other cellular enzymes.

RESULTS: ENOTprost was able to enhance the cellular antioxidant defence by increasing the level of SOD1 compared to control cells as showed in the following histogram.



6- Safety and warnings:

Health risks or adverse events as well as drug interaction following the proper administration of the drug have not been recorded so far (2,5); no concerns arise from the few available data on toxicity (5).

The use in pregnancy and lactation is not applicable due to the indication. Safety in children and adolescents has not been established: in the absence of sufficient data, the use of the extract is not recommended.

The recommended intake is 300-400 mg dose 1-2 times daily (5).

7- References:

1- Plants for a future (PFAF) database, www.pfaf.org

2- Gruenwald J. et al. *PDR for herbal medicines*, 4th Ed. Thomson, 2007

3- Andrew Chevallier, *Enciclopedia delle piante medicinali*, Dorling Kindersley Ed., 1997

4- Capasso F, Grandolini G, Izzo AA., *Fitoterapia*, Springer-Verlag Italia, 2006

5- Assessment report on *Epilobium angustifolium* L. and/or *Epilobium parviflorum* Schreb., herba, EMA, 10 March 2015

6- Schepetkin IA et al. *Therapeutic potential of polyphenols from Epilobium angustifolium (Fireweed)*. Phytother Res. 30(8):1287-97, Aug 2016

7- Kaškonienė V, Stankevičius M, Drevinskas T, Akuneca I, Kaškonas P, Bimbiraitė-Survilienė K, Maruška A, Ragažinskienė O, Kornyšova O, Briedis V, Ugenskienė R. Evaluation of phytochemical composition of fresh and dried raw material of introduced Chamerion angustifolium L. using chromatographic,

spectrophotometric and chemometric techniques. Phytochemistry 115:184-93, 2015.

8- Granica S, Piwowarski JP, Czerwińska ME, Kiss AK. *Phytochemistry, pharmacology and traditional uses of different Epilobium species* (Onagraceae): A review. Journal of Ethnopharmacology, 156, 316–346, 2014

9- Carbonell-Capella JM, Buniowska M, Barba FJ, Esteve MJ, Frígola A. Analytical Methods for Determining Bioavailability and Bioaccessibility of Bioactive Compounds from Fruits and Vegetables: A Review. Comprehensive review in food science and food safety. 13 (2): 155-171, 2014

This monograph is intended for informational purposes only and should not be interpreted as specific medical advice. You should consult with a qualified healthcare provider before making decisions about therapies and/or health conditions.

January 2019