evra®

CITRI-COOS®

Bergamot dry extract
25% flavonoids as naringin

- **Hypercholesterolemia**
- **Metabolic syndrome and type II diabetes**
- **Hepatic and visceral steatosis**
- **Silent inflammation**

CITRICOOS® PRODUCT DATA SHEET:

Product code: ASDCTRBC0525000A

Scientific name: Citrus bergamia Risso & Poit.

Synonym: Citrus aurantium var. bergamia (Risso) Brandis.

Common name: bergamot

Drug: fruit.

Extraction solvent: water / ethanol.

Supporting excipient: maltodextrin.

Origin of the drug: Italy (Calabria).

Country of production of the extract: Italy.

Production plant: Evra srl Benefit Company - Lauria (PZ).

Organoleptic characteristics: fine powder with a yellowish to amber color.

Suggested dosage: 500 mg x 2 times a day.

Solubility: partially soluble in water.

Particle size: 90% through 35 mesh (500 µ).

Tapped density : about 0.5 g / ml.

Loss on drying (105 ° C x 3 h): 5% max.

Heavy metals: Pb <3 ppm; Cd <1 ppm; Hg <0.1 ppm.

Residual solvents: compliant with Dir. 32/2009 / EC.

Pesticides: compliant with Reg. 396/2005 / CE and subsequent modifications

Polycyclic aromatic hydrocarbons: compliant with Reg. 1933/2015 / EC.

Aflatoxins: Aflatoxin B1 <5 ppb Total aflatoxins (B1, B2, G1, G2) <10 ppb.

Total bacterial load (TAMC): 50000 CFU / g max.

Total yeasts and molds (TYMC): 500 CFU / g max.

Pathogens: Salmonella absent / 25 g; E. coli absent / 1 g.

Enterobacteriaceae: 100 CFU / g max.

Non-irradiated product.

It does not contain GMOs (Reg. 1829 and 1830/2003 / EC).

It does not contain traces of BSE / TSE or products of animal origin.

Melamine: compliant with Reg. 594/2012 / EC.

It does not contain any of the allergens included in Annex II of Reg. 1169/2011 / EC.

Product free from ethylene oxide residues.

Storage: in a cool place, in the original containers, properly sealed, away from light, humidity and direct heat sources.

Minimum shelf life: 36 months from the date of production.

Product suitable for vegans.

EU food grade certified product.

BIBLIOGRAPHY:

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[9] Choe SC et al. Naringin has antiatherogenic effect with the inhibition of intercellular adhesion molecule-1 in hyperholesterolemic rabbits. J Cardiovasc. Pharmacol. 2001; 38: 947-955.

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[13] Punithavathi VR, Anuthama R, Prince PSM. Combined treatment with naringin and vitamin C ameliorates streptozotocin-induced diabetes in male Wistar rats. J Appl Toxicol 2008; 28:806–13.

[14] Jung UJ et al. The hypoglycemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KsJ-db/db Mice. J Nutr 2004; 134: 2499–503.

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[17] Rondanelli et al. Bergamot phytosome improved visceral fat and plasma lipidprofiles in overweight and obese class I subject with mildhypercholesterolemia: A randomized placebo controlled trial. Phytotherapy Res. 2021; 35:2045–2056.

[18] Pari L, Amudha K. Hepatoprotective role of naringin on nickel-induced toxicity in male Wistar rats. Eur J Pharmacol 2011; 650: 364-70.

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[20] Alam MA et al. Naringin improves diet-induced cardiovascular disfunction and obesity in high carbohydrate high-fat diet-fed rats. Nutrients. 2013; 5: 637-50.

Bergamot is a hybrid of the *citrus* genus of uncertain taxonomy, obtained by crossing bitter orange either with lemon, lime or cedar; its geographical origin is also uncertain: it likely derives originally from Asia or from the Antilles, and was then successfully domesticated in Calabria, in a narrow coastal strip where it has found very favorable pedo-climatic conditions.

Since the 1700s, it has been considered a mere essential species, cultivated to obtain the precious essential oil from the pericarp, used in perfumery and in the food industry for its special aromatic notes, and in pharmaceuticals for its antiseptic properties. The interest in the potential nutraceutical applications of the fruit, juice and related standardized extracts is more recent and subsequent to the phytochemical characterization; the bergamot fruit has a very special phytocomplex, with a distinctive polyphenolic profile compared to others in the *citrus* genus: secondary metabolites of the class of flavanones and flavones stand out for their abundance. Bergamot, its juice, and the corresponding purified polyphenolic fractions have been tested *in vitro* and *in vivo,* highlighting numerous biological actions, both non-specific (such as antioxidant) as well as specifically aimed at well-identified enzymatic and cellular targets (such as statin-like action of some components). In their complexity, they explain in terms of mechanism of action the favorable effects that the drug and its extracts have shown in clinical studies, especially for the treatment of glyco-lipid dysmetabolisms and, more generally, in cardio-vascular prevention.

Citricoos® is a dry extract of the bergamot fruit, standardized to 25% of total flavonoids expressed as naringin. It represents an ideal candidate, alone and in combination with other active ingredients, for the formulation of food supplements intended for the phyto-therapeutic treatment of metabolic syndrome and type II diabetes, dyslipidemia, and hepatic and visceral steatosis.

Scientific name: *Citrus x bergamia* Risso e Poit. Synonym: *Citrus aurantium* var. *bergamia* (Risso) Brandis.

Common name: bergamot.

Drug: fruit.

Origin of the drug: Italy (Calabria).

Country of production of the extract: Italy.

Production plant: EVRA srl Benefit Company -Lauria (PZ).

The phytocomplex of the bergamot fruit: the phytochemical composition has been analyzed by several authors including Russo *et al.* [1], who characterized the various parts (pulp, juice, seeds and rinds) comparing also the profile for two different cultivars, femminello and fantastico, without, however, detecting significant differences. Flavonoids are the most abundant class of compounds, followed by limonoids; the molecules of both groups are in the free and glycosidic form, the latter distributed significantly in the pulp and juice thanks to the water solubility. The phytocomplex also contains phenolic acids (derivatives of caffeic acid) and oxygenated heterocycles (coumarins and furocoumarins) but the flavonoids are mainly responsible for the biological activities. The most represented are flavanones (free, O-glycosylated and esterified O-glycosylated: naringin, narirutin, eriocitrin, neoeriocitrin, neohesperidin) and flavones (free, O- and C-glycosylates: chrysoeriol, luteolin, apigenin, diosmin, neodiosmin, rhifolin). The latter group includes two molecules with 3-hydroxy-3-methylglutaryl terminal on the glycoside neohexperidose, potentially able to bind to HMG-CoA reductase, inhibiting it: melitidin (naringin-O-HMG) and brutieridin (neohesperidin-O-HMG) .

COMPOSITION OF CITRICOOS®

Citricoos® is a dry extract obtained from the bergamot fruit after removal of the epicarp; the extraction of the raw drug is carried out using traditional methods in hydroalcoholic solvent at the EVRA srl Benefit Company plant in Lauria (PZ), a few kilometers away from the citrus growing and harvesting area.

Citricoos® is standardized to 25% flavonoids expressed as naringin and quantified with the HPLC method. A chromatogram of the extract with the identification of the main peaks is shown, as an example, in fig. 2.

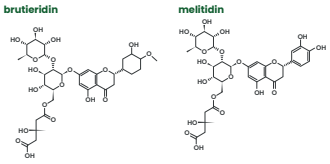

Fig. 1: structural formula of Bergamot HMG-flavones

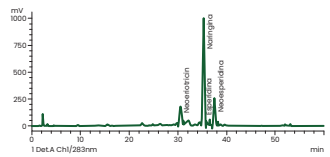

Fig. 2: HPLC chromatogram of Citricoos® bergamot extract

"BERGAMOT FLAVONOIDS REGULARIZE THE PLASMA LIPID PROFILE AND HAVE VASO-PROTECTIVE EFFECT.

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BERGAMOT FLAVONOIDS IN THE REGULATION OF GLYCEMIA.

BERGAMOT FLAVONOIDS REGULARIZE THE PLASMA LIPID PROFILE AND HAVE VASOPROTECTIVE EFFECT.

- inhibition of HMG-CoA reductase, ACAT and other enzymes of lipid synthesis
- modification of lipoproteins
- activation of AMPK
- induction of eNOS, inhibition of LOX-1 and ICAM-1 adhesion protein
- antioxidant action

Bergamot flavonoids have shown cholesterol-lowering and lipid-lowering efficacy in numerous preclinical and clinical studies performed both on fresh juice and on standardized extracts, from the juice and from the fruit without pericarp. The effect is due to a multi-target mechanism of action that mainly involves flavanones. Naringin, the most abundant, inhibits hepatic HMG-CoA [1], as could brutieridin and melitidin, which, according to molecular models, possess a 3-hydroxy-3-methylglutaryl chain that would allow them to bind to the active site of the enzyme nhibiting the binding of the endogenous substrate and negatively interfering with the rate-limiting step of cholesterol neo-synthesis [2]. It is very likely that this statin-like action, evident also *in vivo*, is however the result of the synergy of several flavonoids (not only the 3-HMG-derivatives) on different targets and is not only due to the competitive inhibition of the enzyme cited. Naringin, neoeriocitrin, apigenin, rutin and other bergamot flavonoids can inhibit hepatic and adipocyte phosphodiesterases and, indirectly, activate AMPK (a sort of general switch of energy metabolism); some of the many cellular effects are: the non-competitive inhibition of HMG-CoA reductase and AcylCoA Co-carboxylase (ACC); a reduced activation of SREBP1 (Sterol Response Element Binding Protein 1), a transcription factor that controls lipogenesis, and of PCSK9 that slows the recirculation of LDLr (LDL receptor) towards the hepatocyte cellular membrane. This mechanism has been demonstrated both *in vitro* and *in vivo* for many flavonoids, including those contained in the extracts of fruits of the genus *Citrus* [3]. Mollace *et al.* [4] showed that the administration of 500-1000 mg / day for 30 days of a purified polyphenolic fraction of bergamot (BPF) in subjects with dyslipidemia reduces total and LDL cholesterol accompanied by a lower urinary excretion of mevalonic acid, a marker of HMG-CoA reductase activity.

According to the aa, BPF could be an ideal nutraceutical addition to conventional statin therapy, which was confirmed in a clinical study on hyperlipemic patients already being treated with rosuvastatin [5] in whom BPF provided an additive effect in lowering: plasma levels of total and LDL cholesterol, the LDL to HDL ratio and the expression of lectin-like oxyLDL receptor-1 (LOX-1), simultaneously increasing PKB phosphorylation in polymorphonuclear cells taken from peripheral blood. These last two molecular events mentioned are indicative of a lower atherogenic risk. Patients treated with bergamot in addition to the drug also showed a further decrease in mevalonate, compared to those treated with statin alone.

In a mouse model of hyperlipidemia, oral administration of BPF favorably modified the composition of plasma lipoproteins and counteracted vascular redox stress, increasing the concentration of HDL and reducing LDL and IDL. At the same time, the activities of: ACAT (AcetylCoA Acetyl Transferase), CETP (Cholesteryl Ester Transfer Protein), LCAT (Lecithin Cholesterol Acyl Transferase) and other enzymes that regulate the assembly of lipoproteins were reduced. Simultaneously, concentrations of apoB increased and those of apoA1 decreased [6].

In animal studies and clinical research, bergamot extracts have reduced plasma triglycerides (TG) in addition to cholesterolemia, modifying another important cardio-vascular risk factor. This is what emerged, for example, in the aforementioned study by Mollace *et al.* [4]; by administering BPF to patients with isolated or mixed hypercholesterolemia, in addition to obtaining a significant reduction in total (average -28.1%) and LDL (average - 33.2%) cholesterol, they also recorded a decrease in TG (average -41%) and a parallel increase in HDL (average +30.3%). All the receptor and enzymatic mechanisms of the lipid-lowering effect of bergamot extracts are well synergized by the powerful direct and indirect antioxidant action; in fact, polyphenols act as a scavenger of oxygen (ROS) and nitrogen (RNS) free radicals and stimulate the endogenous antioxidant defense. *In vitro*, naringin and its aglycone effectively block hydroxyl and superoxide radicals and inhibit xanthine oxidase [7-8].

At the vascular level, bergamot polyphenols induce eNOS, counteract the genesis of foam-cells and the consequent formation of atheromatous plaque, protect the endothelium, decrease the expression of the adhesion protein ICAM-1 and increase flow-mediated vasodilation. [9-10]. *In vivo* BPF contrasts the formation of neointima with proliferating muscle cells in an animal model of vascular damage induced by carotid angioplasty [11]; this effect depends significantly on the antioxidant action, as demonstrated by the reduced concentrations of nitrotyrosine measured in the tissues of the treated animals compared to those found in control animals.

In subjects with dyslipidemia, who have a baseline dysfunctional endothelium-mediated vascular response, the administration of BPF significantly improved reactive vasodilation of the brachial artery [4].

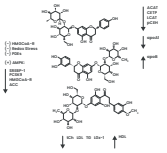

Fig. 1: Main mechanisms of lipid-lowering action of bergamot. (-) inhibition; (+) activation.

PDE (phosphodiesterase); AMPK (AMP-activated protein kinase); SREBP (Sterol Response Element Binding Protein 1); PCSK9 (Proprotein Convertase Subtilisin/Kexin type 9); HMGCoA-R (Hydroxy Methyl Glutaryl CoA reductase); ACC (Acyl CoA Co-carboxylase); ACAT (Acyl-CoA: cholesterol acyl Transferase); CETP (Cholesteryl Ester Transfer Protein); LCAT (Lecithin – Cholesterol Acyl Transferase); pCEH (pancreatic Cholesteryl Ester Hydrolase); tCh (total Cholesterol); LDL (Low Density Lipoprotein); TG (triglycerides); LOX-1 (lectin-like oxyLDL receptor-1); HDL (High Density Lipoprotein); apoA1 e apoB.

BERGAMOT FLAVONOIDS IN THE REGULATION OF GLYCEMIA.

- increase in cellular glucose uptake
- activation of AMPK
- improvement of insulin sensitivity and glucose tolerance

The activation of AMPK operated by bergamot flavonoids also has positive effects on carbohydrate metabolism, improves insulin sensitivity and glucose tolerance, and optimizes glycemic control. When activated by phosphorylation, this kinase triggers energy metabolism by facilitating the uptake of glucose via the GLUT 1 and GLUT4 transporters, blocking adipogenesis and stimulating the β-oxidation of fatty acids.

In vitro, naringenin effectively induces phosphorylative activation of AMPK increasing the influx of glucose into cell cultures of L6 skeletal muscle cells (by facilitating the translocation of GLUT-4 on the plasma membrane) [12]. This action is not suppressed by SIRT-1 inhibitors, therefore it is plausible that the hypoglycemic effects of bergamot are not mediated through the latter enzyme, as is the case with resveratrol.

The co-treatment with naringin (30 mg / kg) and ascorbic acid (50 mg / kg) of streptozocin-diabetic rats improves insulinemia and prevents excessive oxidative stress in the animals of the experimental group compared to the controls. In *db/db* mice, the dose of 0.2 g / kg reduces insulinemia, normalizes pancreatic β cell histology, inhibits glucose-6-phosphatase and phosphoenolpyruvate-carboxykinase significantly more than in the control groups [13]. Improvements in insulin resistance and glucose intolerance have also been noted in studies on animals treated with naringin and naringenin simultaneously with the administration of high-fat diets.

These evidences are consistent with what emerged in the aforementioned clinical study by Mollace *et al.* [4], in which hyperlipemic subjects treated with BPF had, among other effects, a decrease in blood glucose (- 18.9% with 500 mg / day and

-22.4% with 1000 mg / day) which did not manifest in those of the placebo group.

Rutin, which is part of the bergamot phytocomplex, also has a hypoglycemic activity related to the activation of AMPK in pancreatic β cells and hepatocytes; it attenuates the metabolic syndrome induced in rats by an unbalanced "western" diet. Hesperidin, administered to diabetic *db/db* mice, regulates the hepatic metabolism of glucose by acting on glycolytic and gluconeogenetic enzymes, improving hyperglycemia [14]. This flavonoid stimulates the activity of hepatic glucokinase and induces PPARγ and the translocation of GLUT-4 [15].

BERGAMOT POLYPHENOLS FIGHT

Nonalcoholic Fatty Liver Disease (NAFLD).

- stabilization of the hepatocyte membrane
- antioxidant and anti-inflammatory action
- inhibition of fat accumulation and of fibrotic process

Non-alcoholic fatty liver disease (NAFLD) is an almost inevitable clinical element of the corollary that characterizes the metabolic syndrome. Fatty degeneration of the liver, with consequent chronic inflammation, can cause a progressive degree of fibrosis up to liver cirrhosis and hepato-carcinoma, which are extreme consequences. According to some preclinical and clinical studies, bergamot could counteract the onset and evolution of this condition, which has a high incidence especially among populations with unbalanced, typically Western diets. The potential protective effect of the polyphenolic fraction of bergamot BPF99 was studied in a mouse model of NAFLD that mimics the clinical situation in humans very well, including the evolution into nonalcoholic steatohepatitis (NASH) [16]. A group of mice was randomized to receive a normal diet or a steatogenic diet (with the possibility of drinking sugared water *ad libitum*) in the presence and absence of co-treatment with bergamot formula (50 mg / kg for 16 weeks). In the animals of the experimental group, compared to those of the control group, the herbal medicine significantly improved insulin sensitivity and glucose tolerance, slowed the rise of Alanine aminotransferase (ALT) and afforded a clear antihyperlipemic effect. In the liver, it reduced the incidence of NASH compared to untreated animals (3/10 vs 8/10 in the positive control group) and improved the disease histological score. At the biochemical level BPF99 inhibited the phosphorylation of JNK / p38 MAPK and PARP (Poly ADP Ribose Polymerase); there was also a strong decrease in oxidative stress and in the biosynthesis of procollagen-I (anti-fibrotic action). All these biochemical events concur to curb the inflammatory and fibrogenic processes characteristic of NASH.

The pre-clinical results were also confirmed in a clinical study that evaluated the effect of administering a phytosomal BPF in 64 overweight or obese subjects with medium-grade hypercholesterolemia, randomized to receive 2 x 500 mg / day for 12 weeks of BPF-phytosome or corresponding placebo [17]. Among the outcomes there were both the evaluation of the plasma lipid profile and the measurement of visceral fat with DXA *(Dual Energy X-ray Absorptiometry)*. Visceral fat (at all levels, not just in the liver) is a very active tissue from the metabolic aspect; it is considered a reliable indicator of cardiovascular and even neoplastic risk and is strongly correlated with the degree of silent inflammation. Already after 30 days of administration, the experimental group of patients achieved a significant reduction in visceral fat compared to those who took

The hepatoprotective effect of naringin has been reported by several authors: flavanone reduces transaminases in a murine model of hepatotoxicity induced in rats by cadmium and nickel (respectively with flavonoid doses: 50 mg/kg and 80 mg/kg); it reduces the peroxidation of hepatic lipids and induces the endogenous enzymatic antioxidant defense system (SOD, Gpx, CAT, GST) [18-19]. In other studies, inhibition of collagen accumulation and smooth muscle cells proliferation in the liver was also found.

Naringin, administered to rats under a steatogenic diet, prevents the elevation of hepatic markers in plasma, the deposition of fat in the liver and fibrosis [20].

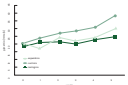

Fig. 2: effect of naringin and hesperidin on blood glucose, in *db/db* mice. Modified from [14].

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