

OLEASELECT™

A HIGHLY STANDARDIZED
OLIVE FRUIT EXTRACT





BOTANY

Olea europaea L. (Olive) of the Oleaceae family is a small evergreen tree, widely cultivated in the Mediterranean countries already from the remote antiquity.

According to De Candolle it is native of the Middle East (Syria and Palestine) but new theories consider the cultivated plant as descending from the wild variety, very common in Italy nowadays as in Pliocene period.¹

The tree attains a venerable age but seldom exceeds 10 meters in height. It has a broad crown and a thick trunk, sometime a shrub; bark grey, finely

fissured; twig lepidote, grey; buds very small, lepidotesericeous, greyish. The leaves are from 20 to 80 mm long and from 5 to 15 mm wide. They are subsessile, mucronate, dark greyish-green, and glabrous above, light grey and densely lepidote beneath. The flowers are arranged in many-flowered axillary or terminal racemes, sub-rotate and 4-lobed. The fruit is an oleaginous drupe from 10 to 35 mm long and from 6 to 20 mm wide. They are ellipsoid to subglobose, green when unripe, becoming black or brownish-green.²

The olive tree is the symbol of the Mediterranean civilisation, which has been accompanied through the centuries by this ancient plant. It used to provide the Mediterranean populations with nutrients, light, heat and medicinal health remedies, creating in the people a very special relationship with the olive tree. Several myths and legends are based on olive: it is a world wide recognised symbol of peace, it was said to be immortal as it is extremely long living, it is mentioned in the Bible. In the Greek mythology it was said to be created by Athena, the goddess of Wisdom, as the most useful invention for mankind; another legend narrates that the olive plant was born on Adam's tomb from a seed coming from terrestrial paradise.

Ever since ancient times, both Pliny and Hippocrates prescribed medications containing olive oil and olive leaves as cures for a number of disorders, ranging from inflammation of the gums to insomnia, nausea, and boils. Many of these old remedies have passed into folk medicine and are still as relevant today as they were hundreds of years ago. More recently, the beneficial health properties of olive oil consumption have been correlated to a decreased incidence of cardiovascular diseases and certain cancers in the Mediterranean area.

OLEASELECT™ PROFILE

OLEASELECT™ is a patented olive pulp extract from a selected Italian variety of olive, particularly rich in polyphenols. It is obtained through an innovative extraction procedure using water and ethanol as the only solvents. Forty to sixty-two kg of olive fruits have to be

processed to obtain one kg of OLEASELECT™ (E/D ratio 1:40-62). The standardized olive pulp extract, purified according to Indena method, contains not less than 30% of polyphenols. OLEASELECT™ specifications are reported below (Table 1):

Table 1 OLEASELECT™ specifications

Total Phenols (UV) ≥ 30%	Phenols (HPLC)	
	HYDROXYTYROSOL ≥ 1.5%	VERBASCOSIDE ≥ 5.0%



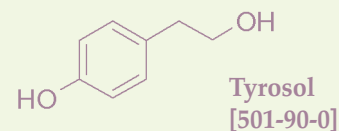
BIOLOGICAL PROPERTIES

The hypothesis that components of olive oil other than $\omega 3$ and $\omega 6$ fatty acids greatly contribute to its benefits on human health,³ stimulated researchers to investigate the biological profile of the other olive components. Numerous studies have shown that phenols are potent inhibitors of LDL oxidation *in*

vivo.^{4,5} The *in vivo* oxidation of LDL is linked to the formation of atherosclerosis plaques, which is known to contribute to the development of coronary heart diseases.

Among the polyphenolic compounds (Fig. 1, 2, 3), **verbascoside** and **hydroxytyrosol** are the most important

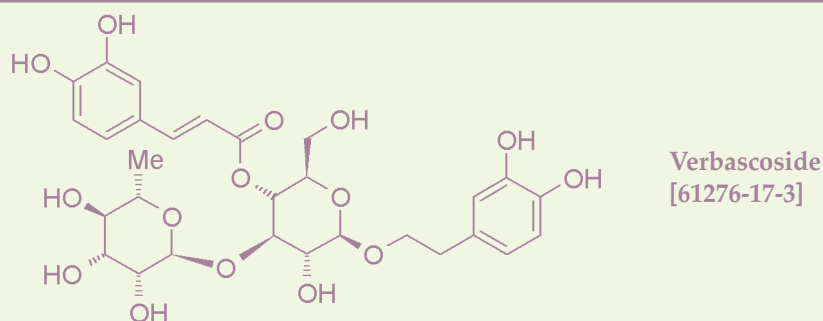
Fig. 1 The structure of Tyrosol



reported to be responsible for many of the biochemical and biological effects of the extract.

VERBASCOSIDE (VB), has a vast distribution in plant kingdom, and in the olive tree is present in the olive pulp, but not in the leaves. Most of the plants containing VB as a major constituent are traditionally used in the treatment of allergic and inflammatory diseases.⁶⁻⁹ More recently VB has been described to exert interesting biological properties:

Fig. 2 The structure of Verbascoside



ANTIOXIDANT ACTIVITY

Several experimental data revealed:

- A scavenging action on superoxide anions and hydroxyl radicals.¹⁰⁻¹²
- The inhibition of the peroxidation of liver microsome and mitochondria in rodents, and the emolysis of red blood cells (RBC) induced by radicals.
- A reduction of oxidative stress in muscle due to exhaustive exercise by decreasing the concentration of free radicals and the level of lipid peroxidation.¹³

NEUROPROTECTIVE EFFECTS

Treatment with VB attenuated apoptotic death and oxidative stress of PC12 neuronal cells line induced by MPP⁺, increased extracellular hydrogen peroxide level, decreased the activation of caspase-3 and the collapse of mitochondrial membrane potential. VB may be useful in reducing neurodegenerative diseases induced by oxidative stress.¹⁴

ANTICANCER ACTIVITY

- Inhibition of protein kinase C,¹⁵ topoisomerase II and telomerase.^{15,16}
- Induction of apoptosis in promyelocytic leukemia HL 60-cells and reversion of malignant phenotypic characteristic on human gastric adenocarcinoma cell line.¹⁷

ANTI-INFLAMMATION ACTIVITY

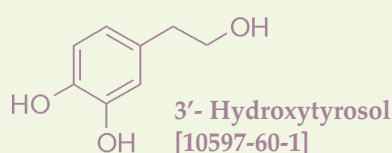
- VB shows anti-inflammatory profile acting as selective inhibitor of 5-lipoxygenase.¹⁵

HYDROXYTYROSOL (HT) one of the major phenolic compound present in olives and olive oil, has been reported to have:

ANTIOXIDANT AND RADICAL SCAVENGING ACTIVITY

- HT reduces the risk of coronary heart diseases and atherosclerosis being *in vitro* a potent and dose dependent inhibitor of copper sulfate-induced oxidation of LDL.¹⁸⁻¹⁹

Fig. 3 The structure of Hydroxytyrosol



- HT acts as antioxidant and metal chelator in animal models.^{5,20,21}
- HT effectively scavenges superoxide anion generated by either human

polymorphonuclear cells or by xanthine/xanthine oxidase system.²²

- HT protects toward hydrogen peroxide-induced damage in human erythrocytes.²³
- HT (50 μ M) prevents DNA damage and tyrosin nitration induced by peroxynitrite.²⁴⁻²⁵
- In *in vivo* studies, low doses of HT, (414 μ g/rat) are able to inhibit passive smoking-induced oxidative stress.²⁶

ANTI-INFLAMMATION ACTIVITY

- HT has proven to inhibit chemically induced platelets aggregation, the

accumulation of the pro-aggregant agent thromboxane in human serum, the production of the pro-inflammatory

leukotrienes by activated human leukocytes, and to inhibit arachidonate lipoxygenase.²⁷⁻³⁰

BIOLOGICAL PROFILE OF OLEASELECT™

The antioxidant activity of this extract was evaluated in cell-free and cell-based experimental models.

CELL-FREE MODELS

The following parameters have been assessed:

- 1) ability to quench the stable free radical DPPH;³¹
- 2) antilipoperoxidant activity (ORAC assay), using Trolox, the water soluble analogue of vitamin E as reference compound³² (values expressed as µg/mL Trolox equivalents);

CELL-BASED MODELS

- 3) ability of protecting the rat RBC membrane lipid peroxidation induced by cumene hydroperoxide (CuOOH) evaluated as inhibition of RBC emolysis;³³
- 4) protective activity on human umbilical vein endothelial cells against AAPH oxidative damage.

Cell-Free Models

1. DPPH assay

The free radical scavenging activity of OLEASELECT™ was determined by measuring the hydrogen-transferring ability to the stable free radical DPPH, using verbascoside, caffeic acid and hydroxytyrosol as reference compounds. The results, reported in Table 2, indicate that OLEASELECT™ possess a significant DPPH quenching ability, and considering the total

Table 2 DPPH assay

Compound	IC ₅₀		
	µM	µg/mL	µg polyphenols/mL
Caffeic acid	15.68±0.37	2.82±0.07	-
Hydroxytyrosol	12.72±0.68	1.96±0.11	-
Verbascoside	5.45±0.04	3.40±0.03	-
OLEASELECT™	-	6.92±0.17	1.11±0.03

Values are mean ±SD of 4 independent experiments

polyphenols content in OLEASELECT™, it results much more active than the

single reference compounds.

2. ORAC assay

The antioxidant activity was then confirmed by using the ORAC assay, which evaluates the radical scavenging activity of the tested compounds towards peroxy radicals generated through the thermal decomposition of a radical initiator (AAPH).

In Table 3 the results clearly indicate a greater antilipoperoxidant activity for OLEASELECT™, referring to the true total polyphenols content. This is due to a cooperative

Table 3 ORAC assay

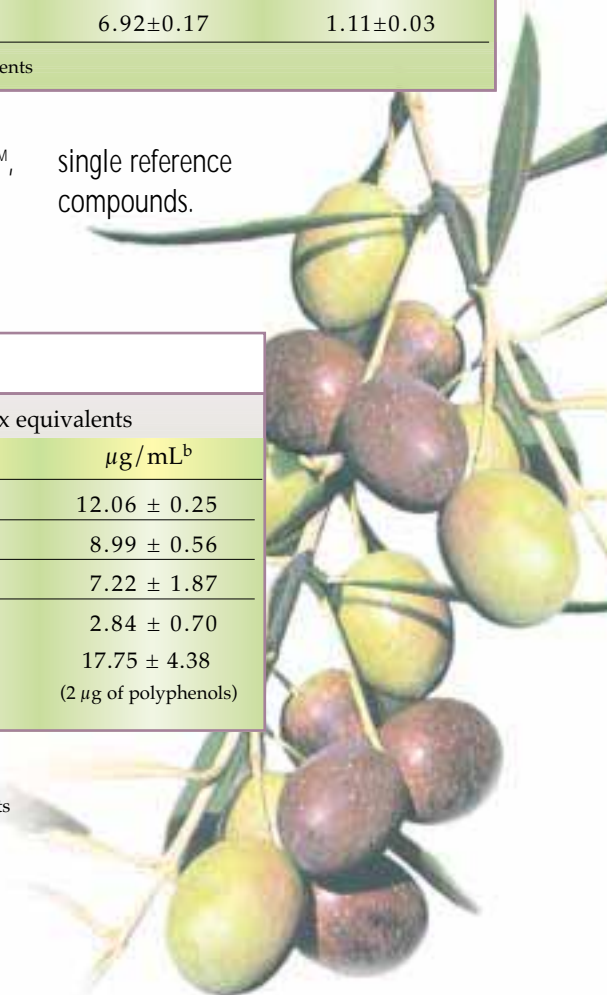
Compound	Trolox equivalents	
	µM ^a	µg/mL ^b
Caffeic acid	25.70 ± 1.17	12.06 ± 0.25
Hydroxytyrosol	21.44 ± 0.52	8.99 ± 0.56
Verbascoside	45.07 ± 8.58	7.22 ± 1.87
OLEASELECT™	-	2.84 ± 0.70 17.75 ± 4.38 (2 µg of polyphenols)

^aµM Trolox equivalents / 5 µM sample

^bµg Trolox equivalents/2 µg/mL sample (powder)

Values are mean ±SD of 4 independent experiments

antioxidant interaction among its components.^{34,35}



Cell-Based Models. Antilipoperoxidant Activity

3. Protective effect on RBC hemolysis induced by CuOOH

As shown in Figure 4 and Table 4, OLEASELECT™ markedly and dose-dependently protected RBC from CuOOH induced hemolysis. At the highest dose the hemolytic process was delayed by 1 hour maintaining RBC intact for 120 min. At 150 min, when submaximal effect has been achieved and in the control sample hemolysis occurred approximately in 100% of RBC, the sample treated with OLEASELECT™

at 0.8 µg/mL the hemolysis occurred in less than 20% of RBC.

4. Protection of human umbilical vein endothelial cells against AAPH oxidative damage.

In Fig. 5, a dose dependent effect of OLEASELECT™ in the protection of human umbilical vein endothelial cell damage by radical inducer AAPH has been observed. The effect on cell viability of OLEASELECT™ was rather identical to the pure compound Verbascoside.

Taking into account the total polyphenol content, OLEASELECT™ is as active as pure verbascoside, thus suggesting a cooperative/synergistic antioxidant effect of some other polyphenol constituents. The relative morphological alteration observable with phase contrast microscopy reveals (Fig. 6) that OLEASELECT™ preincubation greatly reduced alteration induced by AAPH (as shown in Fig. 6 panel C) abolishing almost completely lipid peroxidation and loss of cell viability.

Fig. 4 OLEASELECT™ Protective effect on RBCs hemolysis induced by CuOOH.

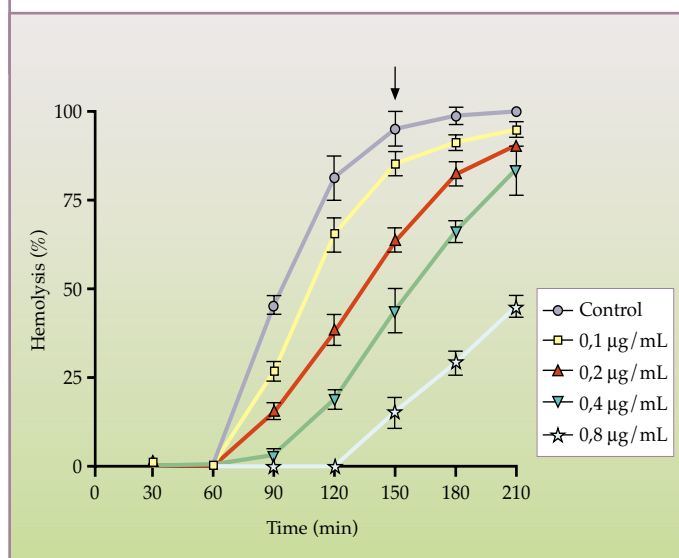


Table 4 IC₅₀ calculated after 150 min of incubation

	µg/mL	µg pol./mL
OLEASELECT™	0.38 ± 0.03	0.06 ± 0.01

Values are mean ± SD of 4 independent experiments

Fig. 5 OLEASELECT™ Dose-dependent protection of human umbilical vein endothelial cells against AAPH oxidative damage

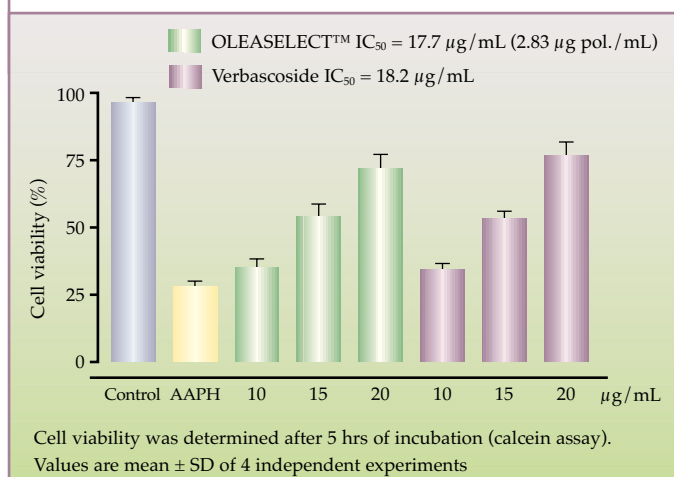
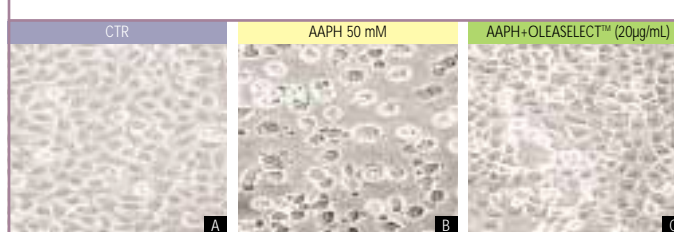


Fig 6 Phase-contrast microscope analysis



TOXICOLOGICAL EVALUATION

The toxicological evaluation showed that OLEASELECT™ is a safe product:

- Acute oral toxicity: non toxic
- Primary skin irritation: non irritant
- Acute eye irritation: non irritant
- Skin sensitization: non sensitizing
- AMES test: non mutagenic

CONCLUSIVE REMARKS

OLEASELECT™ is an extract of olive fresh pulp obtained from a selected Italian variety of *Olea europea*, particularly rich in polyphenols.

From the Mediterranean tradition a highly standardized extract effective in preventing oxidative stress and radical damage with well established health benefits.

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