



Double standardization
in olive polyphenols and
verbascoside

.....
Clinical and
pharmacological data
support its efficacy

.....
Specifically designed
for topical and oral
formulations

.....
A safe product, devoid of
any side effect



Olea europaea L.

The olive tree (*Olea europaea L.*) is an iconic symbol of the Mediterranean area, and since ancient times products derived from its fruit have been used both as a food and as a medicine.^[1,2]

Phytochemical studies have demonstrated that olive oil, olives and processed olives are a rich source of phenolic compounds^[3-6] with potent free radical-scavenging properties.^[7-11]

The Mediterranean diet, which is high in olive oil, fruits, vegetables, grains and legumes, is associated with reduced rate of coronary heart disease^[12-14] and a reduction in all-causes of mortality.^[15]

Furthermore a strong reduction in cardiovascular heart diseases has been associated to a high intake of olive oil.^[16]

The cardiovascular health activity of olive consumption goes substantially beyond those expected from the high content of monounsaturated fatty acids found in olives and has been related to the phenolic compounds present in olives.^[12-17]

More specifically many of these recent clinical studies, comparing olive oils rich or deprived in polyphenols, have underlined how many anti-inflammatory markers might be at the basis of these processes.^[12,14,18]

These findings are backed up by epidemiological data^[19] suggesting that the free radical-scavenging activity of olive polyphenols may also have a direct impact on skin health, including the prevention of oxidative damage related to wrinkle formation, skin-thinning and dehydration.

Olive polyphenols and health claims

FDA has recommended a daily consumption of 23 g of olive oil as part of a healthy diet^[20] and the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) has confirmed the relationship between the daily intake of 5 mg of olive oil polyphenols (standardized by the content of hydroxytyrosol and its derivatives) and the protection of LDL from oxidative damage.^[21]

Opextan[®] specifications

Opextan[®] is a safe^[22] non-caloric standardized olive polyphenolic extract (≥10% of total olive polyphenols and ≥2% of verbascoside) derived from the olives pulp.

Opextan[®] is obtained by a selected variety of olive possessing a unique polyphenolic profile: it contains verbascoside, a secoiridoid conjugate of hydroxytyrosol, a polyphenol characteristic of the olive fruit which is not present in the leaves, and that has been reported as the most potent antioxidant from the olive fruits and leaves.^[3]

Verbascoside is accompanied by minor analogues, as well as by unconjugated hydroxytyrosol and tyrosol.^[23]

Furthermore, Opextan[®] contains ca. 4% citric acid and ca. 6% as elemental potassium.

PHENOLIC COMPOUND	NOMINAL CONTENT (w/w by HPLC)
Verbascoside	2.0 - 3.5%*
Total content of hydroxytyrosol and derivatives	≥ 4.5% ^[23]

Diet and olive polyphenols

The link between olive polyphenols and cardiovascular health has been widely established^[12-17] with FDA recommending the daily consumption of 23 g of olive oil as part of a healthy diet^[24] and the NDA Panel confirming the relationship between the daily intake of 5 mg of olive oil polyphenols (standardized by the content of hydroxytyrosol and its derivatives) and protection of LDL from oxidative damage.^[21]

In Mediterranean countries, the consumption of olive fruit and olive oil has been estimated to be in the range of 30 to 50 g/day,^[24, 25] and given that the median polyphenol content is between 200 and 600 mg/L for a good extra virgin olive

oil,^[26-28] the FDA and NDA Panel indications are in line with the estimated intake of a balanced Mediterranean diet.

It should be remarked, however, that the daily per person energy expenditure is today 1800 Kcal/die^[29] and that 23 to 50 g of olive oil has an associated energetic burden of over 200 Kcal (200 Kcal to 500 Kcal) almost 1/9 to 1/3 of the average daily requirement, while the caloric intake of the corresponding amount of Opextan[®] in terms of phenolic contents (from 46 g to 300 mg as Opextan[®]) is negligible, since fats have been substantially removed in the extraction process.

Clinical studies by oral route

Opextan[®] is supported by extensive pharmacological data and five clinical trials, three for oral^[30,31,34] and two for topical application:^[30,39] it was proven effective in improving antioxidant defenses and in counteracting the oxidative stress; furthermore Opextan[®] showed to improve fasting blood sugar levels.

■ decrease of skin sensitivity to UV irradiation by oral route

Opextan[®] was administered orally at a dose of 160 mg/day for 4 weeks to 13 male subjects in a placebo controlled trial.^[31,32] The subjects were irradiated by ultra violet light (UVA + UVB 0.45 mW/cm²) from 0.054 to 0.135 J/cm² successively on the dorsal area.

The minimum erythmal dose (MED) was measured before and after Opextan[®] treatment. Opextan[®] promoted a mean increase of the MED by 16.45% (p<0.01), suggesting a lower sensitivity of the volunteers' skin to UV irradiation.



Effect of Opextan[®], orally administered, on skin sensitivity to UV irradiation. Images taken before and after the treatment of the same subject.

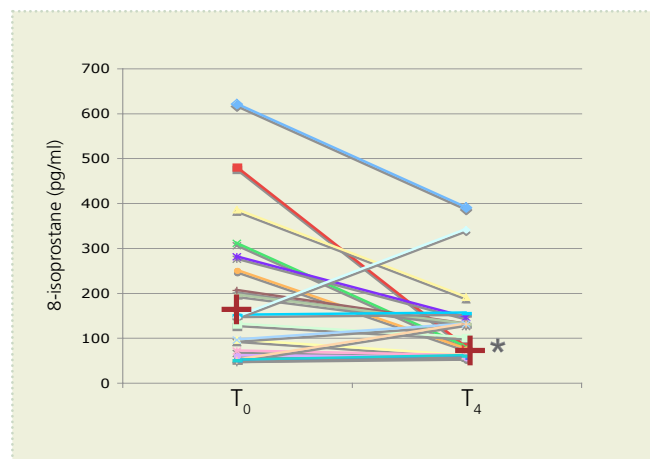
■ improvement of oxidative status in healthy volunteers

Oxidative stress triggers several degenerative disorders and epidemiological studies have suggested that a high intake of dietary antioxidants can effectively counteract it. To evaluate the antioxidant effect of Opextan[®] in volunteers, the *in vivo* peroxidation was evaluated by the excretion of the oxidative marker 8-isoprostane (8-iso-prostaglandin F_{2α}).^[33]

Nineteen healthy volunteers received Opextan[®] at a daily dosage of 400 mg for 4 weeks.^[31,32]

The urinary excretion of 8-isoprostane, as a marker of oxidative stress, was evaluated in urine samples before the beginning of the study and at the end of the 4 weeks' treatment.

Opextan[®] significantly decreased the formation of the oxidative marker (47%, mean data).



Effect of Opextan[®], orally administered, on lipid peroxidation.

■ effect on glucose metabolism on subjects with hypertension^[34]

A further study was carried out on 15 patients under treatment with antihypertensive drugs and with “borderline parameters” for metabolic syndrome^[35] [slightly elevated fasting blood sugar levels (over 110 mg/dL) and blood pressure levels (above 130/85 mmHg)].

The subjects were divided into two groups, and each group was treated with two soft gel capsules a day for 12 weeks. In the first group, each soft gel capsule contained 50 mg

of Opextan[®] and 214 mg of olive oil, while the placebo capsule in the control group contained 252 mg of olive oil corresponding to 0.02 mg of polyphenols.

The blood glucose levels in the treated group decreased significantly, from 116.6 mg/dL to normal fasting level of less than 110 mg/dL (105.8 mg/dL), while no significant modifications in the control group were evidenced.

Pre-clinical studies

The pharmacological profile of Opextan[®] has been defined by *in vitro* and *in vivo* experimental studies. For the *in vivo* studies verbascoside, the typical polyphenol concentrated from the extract, was used.

■ *in vitro*

● antioxidant activity^[31]

Opextan[®] antioxidant activity was evaluated by using the stable free radical DPPH (1,1-diphenyl-2-picryl-hydrazyl). In this assay, Opextan[®] showed an IC₅₀ value of 0.0054% after 20 minutes.

Furthermore, the radical scavenging ability of single polyphenols present in the extract (verbascoside, hydroxytyrosol and caffeic acid) was compared to ascorbic acid and oleuropein (the most characteristic polyphenol present in the olive leaves). Verbascoside turned out to be five times more active compared to oleuropein.

Additionally, Opextan[®] polyphenols were tested for their scavenging activity on superoxide anion O₂⁻, an activated oxygen species. This model mimics closely the *in vivo* situation, since the formation of the superoxide anion takes place in tissues and cells, and is, for instance, triggered by UV exposure of the skin.^[36-37]

Verbascoside was tested at 0.016 mM concentration, and inhibited superoxide anion formation by 68%.

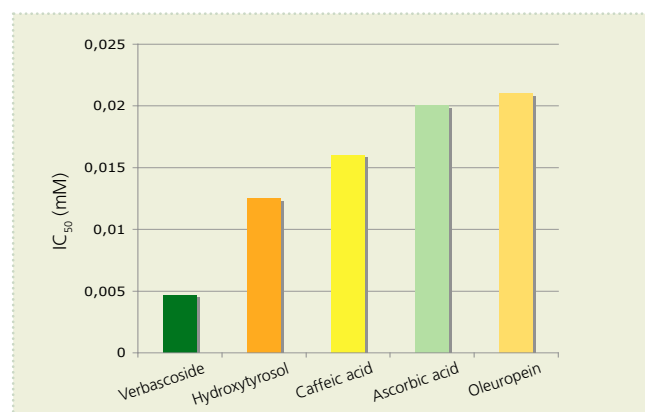
These data are in accordance with an independent study^[2] showing that verbascoside outperforms caffeic acid or hydroxytyrosol, both individually and in equimolar mixtures, in antioxidant assays.

● glucosidase inhibition activity

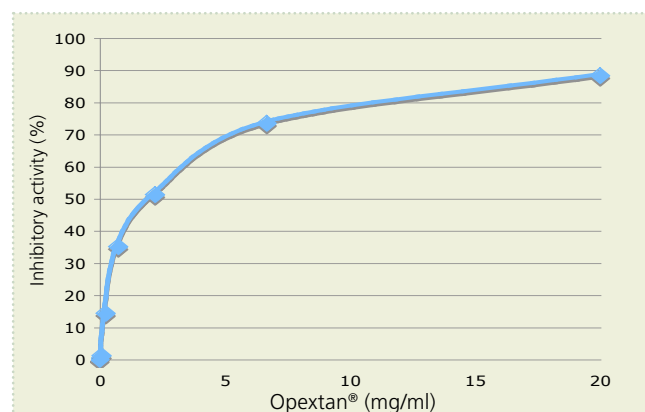
Diabetes can affect every part of the body, including the skin, and the pathology can be complicated by a variety of cutaneous manifestations^[38] that have been also linked to a poor glycemic control.^[39] Blood glucose levels are therefore important also for skin care.

Different concentrations of Opextan[®] were tested for the α -glucosidase activity showing a good inhibition on this enzyme and suggesting one of the potential mechanisms of action for glucose control, which has recently been also assessed in an already mentioned clinical trial.^[34]

Moreover, mixtures of biophenols have been reported to be more active than individual biophenols as antiproliferative agents^[7,8], mixtures of polyphenols being more effective in protecting DNA from oxidative damage than any of their single constituent.



DPPH scavenging activity of Opextan[®] polyphenols.



Effect of Opextan[®] on α -glucosidase activity.

■ **in vivo**

• **hypoglycemic activity**

The hypoglycemic activity and action on insulin levels of Opextan® was evaluated on hairless mice (10 animals)^[31]. Animals were treated with streptozotocin STZ (75 mg/ml/kg intravenously) to induce diabetes.

One week after diabetes induction, the animals were treated with Opextan® (0, 50 or 100 mg/head/day) by oral route for 2 weeks.

Blood glucose levels and plasma insulin were then measured. Results showed that Opextan® was able to modulate plasma insulin towards an increased level and to reduce blood glucose.

	GLYCEMIA (mg/dl)		INSULIN (pg/ml)	
	MEAN	SD	MEAN	SD
Normal	192.99	± 10.74	838	± 82
Control	629.95	± 33.89	55.57	± 55
Opextan® 50 mg	472.21	± 48.77	370.7	± 165
Opextan® 100 mg	456.65	± 49.21	529	± 237

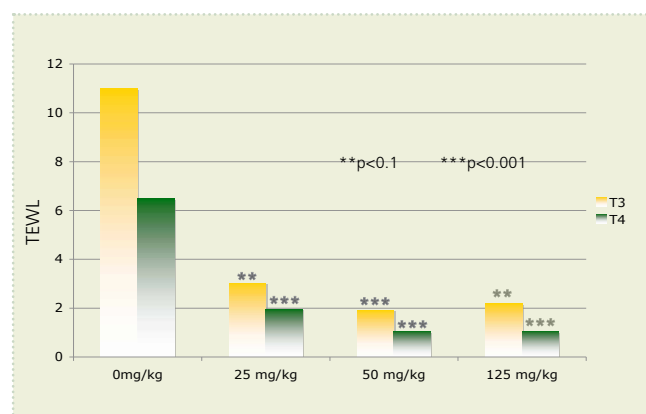
Effect of Opextan® on blood glucose and insulin levels in diabetic mice

• **protection from epidermal permeability barrier**^[31]

UV irradiation induces several cutaneous responses among which disruption of epidermal permeability barrier, measurable by TEWL (Trans Epidermal Water Loss) modification.

Adult hairless mice (4-5 animals per group) were exposed to a single dose of UVB on day 7 of oral treatment with verbascoside. Mice were administered dosages of verbascoside from 125 to 6.25 mg/kg via oral gavage daily for 11 days. TEWL was measured on day 0, day 3 and 4 after UV exposure.

TEWL decreased significantly, thus indicating a protective action exerted by verbascoside.



Effect of verbascoside on UV induced epidermal permeability barrier damages.

• **prevention of UV photoaging wrinkle formation**^[31]

Photoaging and wrinkle formation may be promoted by UV irradiation.

Adult hairless mice (8 animals per group) were exposed to increasing doses of UVA and UVB (starting from 20J UVA and 20J UVB per cm² to reach the doses of 30J and 40J per cm² respectively from week 3 to 12). Irradiation was performed 5 times per week for 12 weeks, with verbascoside dosing beginning on week 5 at 6.25 or 25 mg/kg/day. Wrinkle formation in each animal was determined according to the grading scale as indicated in the table.

Verbascoside, Opextan® most characteristic polyphenol, promoted a prevention of UV induced wrinkle formation by 38% (25 mg/kg dose, p<0.001).

These data have been additionally confirmed by the objective measure of epidermal thickness, which is a useful parameter to evaluate cutaneous inflammation induced by UV. Epidermal thickness was reduced by 28%.

GRADING VALUE	OBSERVED SIGNS
0	no wrinkling or laxity; fine striations running the length of the body
1	disappearance of all fine striations
2	a few deep wrinkles and laxity
3	increased deep wrinkles

Grading scale of wrinkle formation observation.

Clinical studies by topical application

■ reduction of lipid peroxidation by topical application

Lipid peroxidation is a well known example of oxidative damage in lipid rich tissues.^[40]

Lipid hydroperoxides are prominent non-radical intermediates for lipid peroxidation.

The effect of topically applied Opextan[®] was evaluated on a group of 6 healthy volunteers^[31] who, after washing their face, applied a 0.5% Opextan[®] formulation or a placebo formulation on each half face.

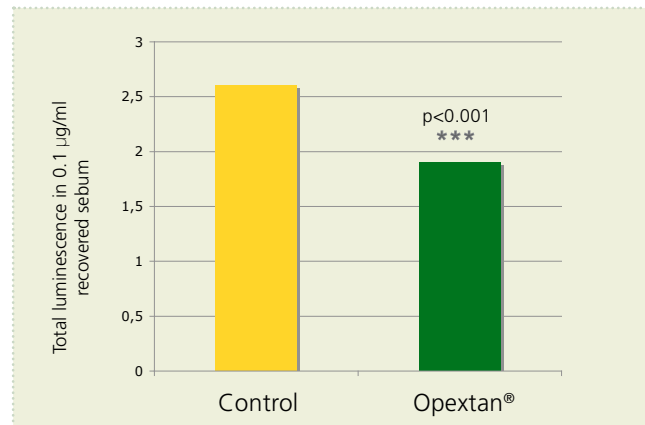
After three hours they were irradiated with sunlight for about 20 minutes. Sebum was sampled, extracted, and measured by dosing luminescence.

Luminescence, as a marker of lipid peroxidation, decreased by 27% in the Opextan[®] treated area.

An additional clinical assessment has been led on 23 male healthy volunteers who applied on each half face the Opextan[®] containing formulation (2.5%) and the blank formulation, twice daily for three months.^[41]

The measurement of moisture of the skin horny layer and the viscoelasticity of the skin, as well as the skin replicas, were performed before the beginning of the study, at week 8 and week 12.

The product containing Opextan[®] showed significant amelioration compared to the placebo group in terms of the roughness parameters of the replica analysis. An ameliorating tendency was seen as to the moisture of the horny layer as well.



Effect of Opextan[®] on lipid peroxidation by topical application.

Conclusive remarks

The antioxidant and radical scavenging capacity of Opextan[®] has demonstrated its beneficial effects at skin level, where free radical mediated degeneration such as wrinkle formation, epidermal barrier disruption, etc., may be effectively prevented.

The effectiveness of the product has been demonstrated both topically and by oral application, suggesting a global approach to maintain a healthy skin by treating it both internally and externally.

What makes Opextan[®] even more unique?

- It is obtained from the edible part of the plant (olives) and not from waste water, as the epidemiological beneficial effects of the olive tree refer to its edible part (fruit pulp and olive oil obtained from it) and not to other plant parts, whose phytochemical profile is different from that of the fruits.
- Opextan[®] is obtained from a single cultivar of *Olea europaea* L., selected for its high contents of polyphenolics.
- Opextan[®] contains a high concentration of the hydroxytyrosol conjugate verbascoside, the most potent antioxidant from the olive tree, while most olive extracts are based on unconjugated hydroxytyrosol, a less potent antioxidant typical of the unedible vegetation waters and of aged extra-virgin olive oils.
- Opextan[®] contains a balanced mixture of polyphenols, that encompasses verbascoside, hydroxytyrosol, tyrosol and caffeic acid, and exploits their synergistic interaction.

Applications and possible formulation:

Opextan[®] is water soluble, and can be easily formulated in:

- Functional drinks
- Functional foods as nutrition bars, etc...
- Tablets
- Soft gel
- Capsules
- Cosmetic topical applications (water solubility)
- In a global approach to inner health and outer beauty Opextan[®] may be formulated both in cosmetics for topical application and oral supplements

Opextan[®] is Halal, Kosher certified and ECOCERT validated.



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