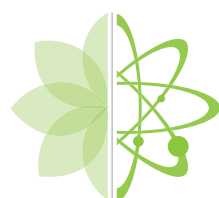


NIO-COMPLEX

revolutionary and powerful
anti-wrinkles system for a broad
spectrum of skin protection



naturalis[®]
LIFE TECHNOLOGIES



ISO 9001
LL-C (Certification)

NIO-COMPLEX

NIO-COMPLEX is a powerful mix of active ingredients of natural origin specially designed to protect skin and to delay the process of skin aging by acting at multiple levels.

NIO-COMPLEX consists of powerful anti-aging natural ingredients such as Quercetin, Morinda Citrifolia seed extract and pure Magnolol/Honokiol extracted from Magnolia bark, carried by niosoma, a novel drug delivery system formed by the self-assembly of non-ionic surfactants in aqueous media in which the active compounds are encapsulated into vesicles.

Cosmetic Use

- Anti-age day and night care
- Active make-up
- Sun care

Main Components

| Active components | Origin | Skin Benefits |
|--------------------|---------------------------|----------------------------------|
| Morinda Citrifolia | Botanical | potent anti-MMP and anti-radical |
| Quercetin | Botanical | Anti-free radical and anti-aging |
| Magnolol/Honokiol | Botanical (Magnolia bark) | anti-inflammatory activity |

INCI/CTFA-Declaration

Quercetin, Morinda Citrifolia Fruit Extract, Magnolol, Honokiol, Polyglyceryl-10 Dipalmitate, Polyglyceryl-6 Laurate, Sorbitan Oleate, Aqua/Water.

Formulating with NIO-COMPLEX

- DOSAGE: 1-3%.
- SOLUBILITY: soluble in water, insoluble in oils or fats.
- MODE OF INCORPORATION: dilute in a part of water before blending in the cosmetic product. Do not heat above 50° C.

SKIN AGING AND METALLOPROTEASES

Dermis primarily consists of an extracellular matrix of connective tissue, whose the most abundant protein is collagen. Chronic UV exposure to the skin results in generation of reactive oxygen species (ROS). This is the first phase of that complex process that leads to degradation of collagen and elastic fibers in the dermis and induces wrinkles (photoaging). In fact, UVA, through the production of ROS, triggers intracellular signal transcription pathways involving over expression of c-Jun and c-Fos, induces the activation of the protein-1 (AP-1) which, in turn, up-regulates expression of metalloproteases (MMPs). MMPs, a family of structurally related matrix degrading

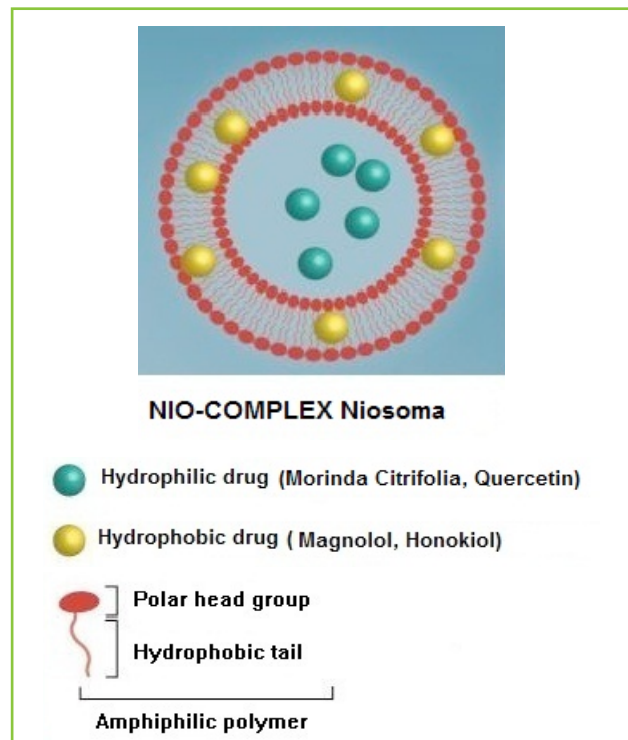
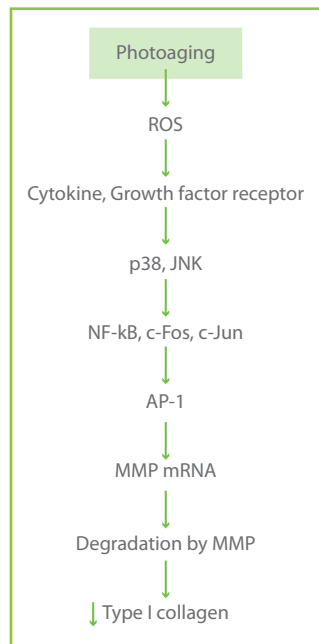
enzymes associated with various destructive processes, degrades collagen and elastin resulting in the formation of wrinkles over time.

NIO-COMPLEX thanks to the synergical combination of Quercetin, Morinda Citrifolia extract and Magnolol, entrapped into niosomal vesicles, represents an effective and innovative active ingredient

to prevent the typical signs of mature skin by neutralization of ROS and inhibition of MMPs .

Niosoma as a potent skin delivery system for effective cosmeceuticals

NIO-COMPLEX is an effective ingredient for skin care formulation in which a potent mix of biologically active molecules are contained into very small vesicles (150-250 nm) known as niosomes. Niosoma is an effective drug delivery system particularly suitable for pharmaceutical and cosmeceutical. It consists of vesicles formed by the self-assembly of non-ionic surfactants in aqueous media in which the active compounds are trapped inside. The niosoma is made of a surfactant bilayer with its hydrophilic ends



exposed on the outside and inside of the vesicle, while the hydrophobic chains face each other within the bilayer.

Like liposomes, niosomes are able to increase drug stability, enhance the effectiveness, prolong circulation time and promote uptake of the entrapped drugs into the target site. However, compared to liposomes, niosomes have additional important advantages such as:

- ability to entrap both hydrophilic and hydrophobic drugs
- greater chemical stability
- no problems related to phospholipids purity
- low toxicity due to non ionic nature

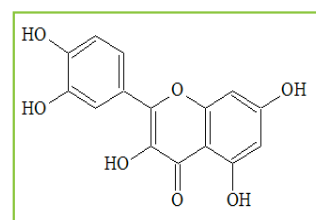
- no requirement of special precautions and conditions for formulation
- production without the use of unacceptable solvents

NIO-COMPLEX increases the stability of entrapped active agents, improves bioavailability of poorly absorbed ingredients and enhances skin penetration improving percutaneous passage of biologically active molecules through human stratum corneum which is known to be a highly impermeable protective barrier.

NIO-COMPLEX: a key inhibitor of skin aging

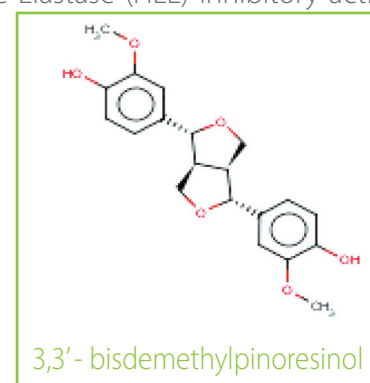
Environmental factors like sunlight, ozone and other pollutants cause excessive levels of Reactive Oxygen Species (ROS) which reduce the skin's resistance and make skin look older than it really is. NIO-COMPLEX is a powerful mix of active ingredients of natural origin specially designed to protect and delay the process of skin aging by acting at multiple levels. In fact NIO-COMPLEX contains: 1) highly purified quercetin, a strong free radical-scavenging bioflavonoid which provide high protection against oxidative injury (ROS protection) and inflammation; 2) Morinda citrifolia seed extract, a strong matrix metalloproteinase-1 (MMP-1) and elastase (HLE) inhibitor 3) Magnolol and Honokiol, two highly purified anti-inflammatory and antioxidant agents obtained from magnolia bark.

- **Quercetin** is a flavonoid found in many common foods. It is also one of the most powerful mo-



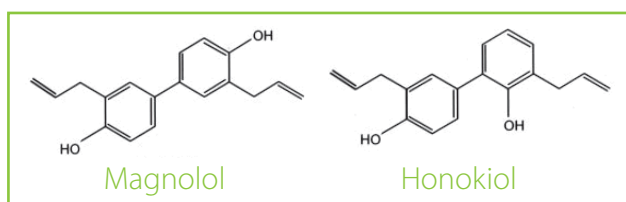
lecules available to cosmetic sciences to combat skin-aging. In fact, Quercetin assists skin renewal by minimising effects of ROS. As well known as an increased ROS levels leads at: 1) the breaking down of the collagen matrix by raising of the levels of matrix metalloproteinases (MMP), 2) wrinkle formations and losing of skin elasticity. Quercetin is also able to block UV irradiation-induced skin inflammation.

- **Morinda citrifolia seed extract.** *Morinda Citrifolia*, known commercially as noni, is one of the most significant sources of traditional medicines among Pacific island societies. It has been demonstrated that *Morinda Citrifolia* seed extracts exhibits both Metalloproteinases (MMPs) and Human leukocyte Elastase (HLE) inhibitory activity. UV induces the production of MMPs by activating intracellular signal transcription pathways, involving the protein



kinase p38 and c-Jun-N-terminal kinase (JNK). Neutrophils play an important role in photoaging, which are set in motion by UV irradiation. These cells infiltrate into the skin and release several active enzymes, such as MMPs and HLE. HLE cleaves the helix structure of type I collagen and then degrades elastic fiber in human skin. HLE also directly activates MMP-1 from human dermal fibroblasts. Morinda citrifolia seeds extract, containing high levels of lignans as 3,3'-bisdemethylpinoresinol that exhibits strong MMP and HLE inhibitory activity, represents an ideal active ingredient to protect skin from photoaging.

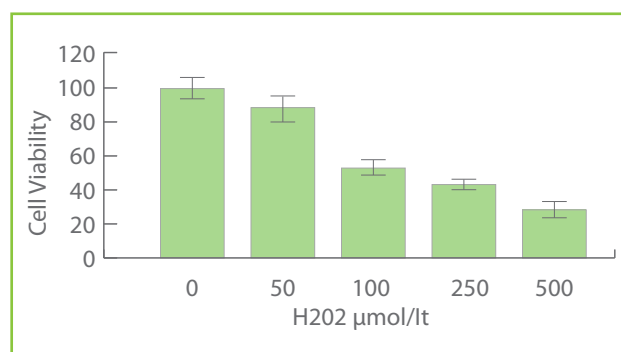
- **Magnolol and Honokiol**, two highly purified hydroxylated biphenyl molecules extracted from magnolia bark, have strong anti-inflammatory and antioxidant properties. They are able to reduce inflammation by inhibiting NF-kB factor through the enzyme inactivation of Ikb kinase (IKK). Thanks the inhibition of the NF-kB factor, Magnolol and Honokiol down regulate also the production of important inflammation mediators as the interleukin-8 (IL-8) and the tumor necrosis factor α (TNF- α).



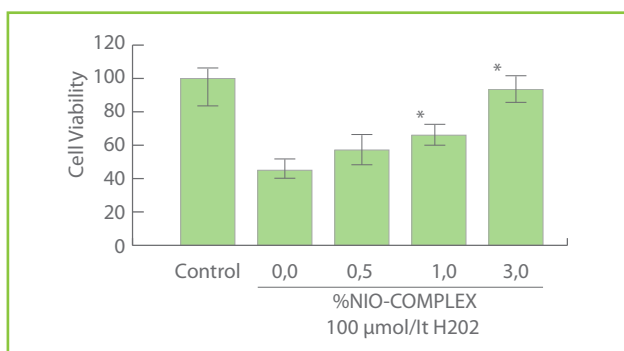
The synergical action of Quercetin, Morinda citrifolia seed extract and Magnolol/Honokiol reduces the visible signs of aging, smoothes fine lines and wrinkles, prevents skin damage. NIO-COMPLEX will make your skin soft, elastic, properly hydrated and comfortable

NIO-COMPLEX: Efficacy tests: Effect of NIO-COMPLEX on H₂O₂-induced oxidative stress in fibroblast cells

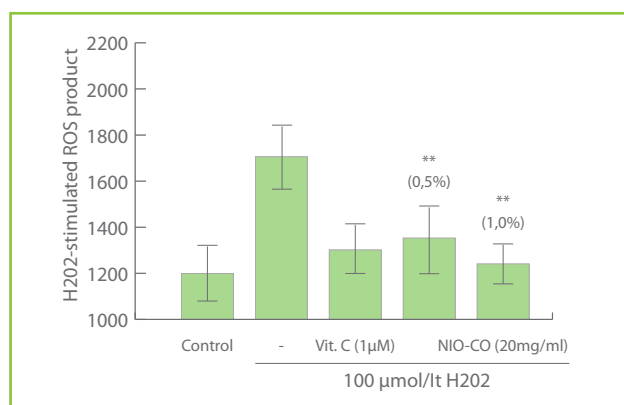
To demonstrate the protective activity against oxidative stress of NIO-COMPLEX, cultured NIH 3T3 cells were pretreated with different NIO-COMPLEX concentrations (0,5-3,0%) overnight followed by 2h of 100 μ M of H₂O₂ stimulation. Afterwards, the cell were washed to remove H₂O₂ and incubate with fresh medium for 48 hours. At the end of this period cell viability was assessed using MTT assay.



Cell viability of fibroblasts NIH 3T3, treated with different concentrations of hydrogen peroxide H₂O₂, significantly decreased in a concentration-dependent manner.*
P<0,01 (Anova test).



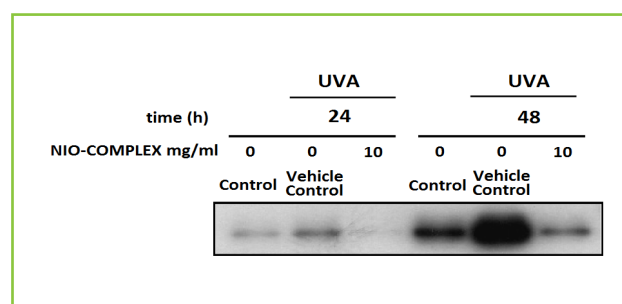
Effect of NIO-COMPLEX on cell viability after treatment with H₂O₂ 100µmol/l. The survival of the cells increased significantly in the cultures pre-treated with NIO-COMPLEX. *P<0,01 (Anova Test).



Antioxidative effect of NIO-COMPLEX in comparison with Vitamin C on H₂O₂-induced oxidative stress in fibroblasts NIH 3T3. DCF Fluorescence was read at $\lambda_{exc}=485\text{ nm}$ and $\lambda_{em}=530\text{ nm}$. **($P<0,05$, ANOVA test.).

All these results demonstrate that NIO-COMPLEX has a remarkable cytoprotective effect against H₂O₂ damage at low concentration. In conclusion NIO-COMPLEX is a very effective active ingredient for skin care formulations able to prevent oxidative damage induced by free radicals and UVA.

Effect of NIO-COMPLEX on MMP-1 secretion in UVA-irradiated fibroblast cells



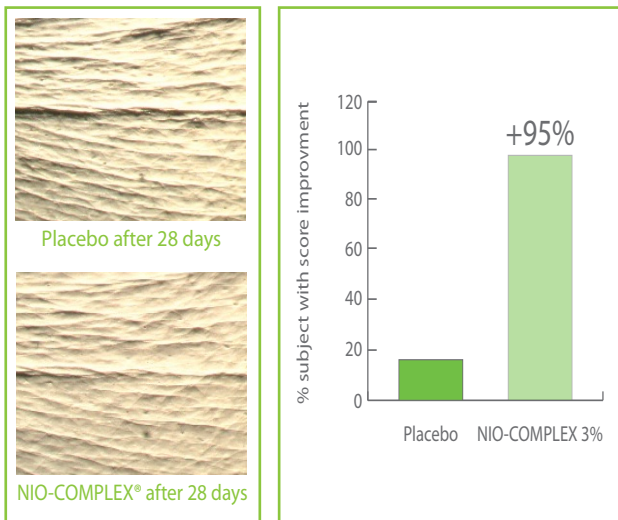
Fibroblasts were exposed to UVA (5J/cm²) and then cultured in the serum free medium containing test samples for 48 h. MMP-1 protein levels in the cultured medium were assessed by Western Blot analysis using antibody anti-MMP-1.

IN VIVO: anti-aging effect of NIO-COMPLEX

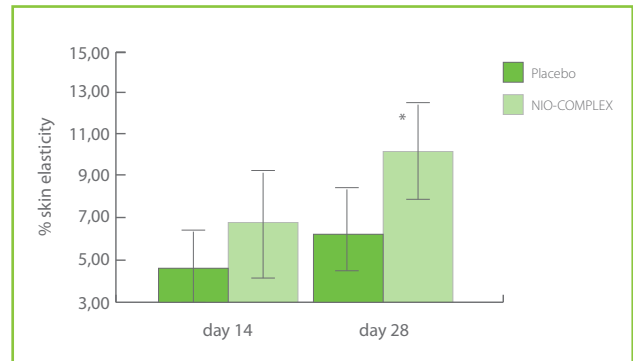
Anti-wrinkle efficacy:

Measurements of "crow's feet" wrinkles imprinting was carried out to study the anti-wrinkle effect of NIO-COMPLEX. In this study 40 volunteers with normal skin and aged from 38 to 65 was divided in two groups and treated for 30 days the first with an oil in water cream containing 3% of NIO-COMPLEX and the second with placebo in double blind. Real-time 3D in vivo measurements to evaluate wrinkle depth was carried out using a light polarized camera connected to a software for the image analysis.

The anti-wrinkles efficacy was assessed a score (0-4) by a basic visual evaluation.

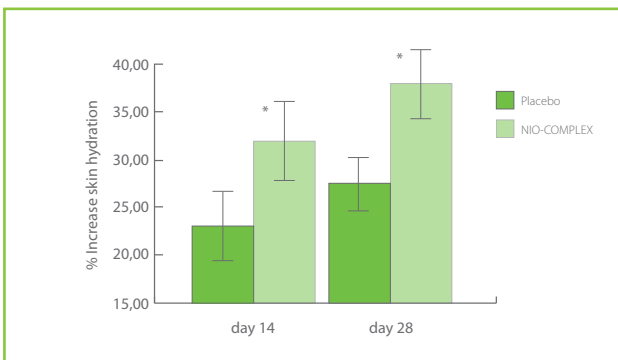


Skin elasticity



NIO-COMPLEX was able to increase also the skin elasticity of the crow's feet after 28 days of treatment. At the end of the test, in the group treated with NIO-COMPLEX the skin elasticity was increased of almost 11% respect to untreated skin indicating an effective role of NIO-COMPLEX in the preventing of skin aging. * $p < 0,05$ (ANOVA test)

Skin hydration:



Results demonstrated that the cream containing NIO-COMPLEX was able to increase skin hydration just after 14 days of treatment. At the end of the test, in the group treated with NIO-COMPLEX the skin hydration increased of 38% more respect to untreated skin. * $p < 0,05$ (ANOVA test).

In summary NIO-COMPLEX:

- shows a strong radical scavenging effect
- increases cell viability after oxidative stress
- reduce ROS production after UVA exposure
- reduce wrinkle depth for 95% of the subjects
- increases skin hydration (+38%)
- improve skin elasticity (+125%).

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