# Leafresh

ECOCERT RAW MATERIAL

COSMOS APPROVED

Skin protection against environmental factors





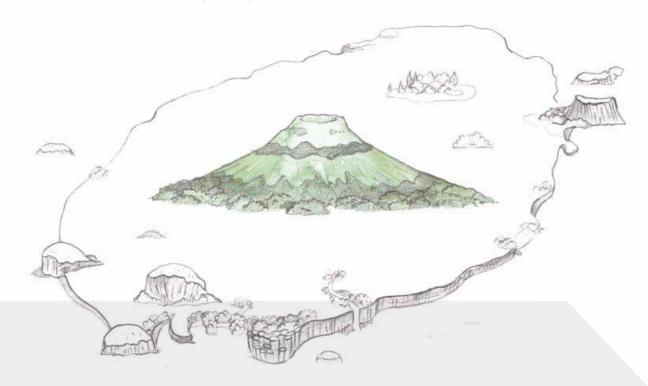
12-24, Gajangsaneopseo-ro, Osan-si, Gyeonggi-do, 18103, Republic of www.natrualsolution.co.kr info@naturalsolution.co.kr

## Jeju Island

Jeju is the largest island situated on the southern coast of Korea.

This island is considered the cleanest and most beautiful area in Korea. It is a volcanic island with Halla Mountain in the center created by volcanic eruptions about 2 million years ago.

Jeju volcanic island and lava tubes are designated as the first World Natural Heritage Sites in Korea. Due to its unique environment, the island has many endemic plants especially in Halla Mountain where there are about 1,800 endemic plant species as well as diverse alpine plants.





### Lotus in Jeju Island



In Jeju Island, there is a lotus pond called 'Yeonhwamot' or 'Yeonhwaji', meaning a lotus flower pond in Korean. This pond is the biggest and deepest pond in the island. The flowers bloom in July and last for a couple of months. People in Jeju celebrate the lotus season with several festivals in the botanical gardens and temples to enjoy the beautiful flowers. One of the famous lotus ponds is in a temple called 'Beophwasa' in Jeju which was built in the 12<sup>th</sup> century.



### **Plant Story**

**Nelumbo nucifera**, commonly known as "lotus," is a large aquatic rhizomatous plant. Its root, seed, leaf, and flower are edible and widely consumed as food or tea in East Asia. The lotus is symbolic of purity and resurrection as the plant rises from muddy water to blossom pure and uncontaminated flower. The lotus is also a symbol of Buddhism, and it is often cultivated in Buddhist temples of Asia. In the temples of Korea, monks harvest the leaves and flowers to consume them as a lotus tea.





### **Plant Story**

The lotus leaf is known to **contain various flavonoids** including rutin, isoquercitrin, hyperin, and quercetin. **Anti-oxidants and UVB protective effects** of the lotus leaf extract were presumably reported due to its flavonoid contents. It was also demonstrated that the methanol extract of lotus flower buds and leaves showed an **inhibitory effect on melanogenesis**.



### **Traditional Uses of Lotus Leaf**



#### TEA

Every part of lotus is used to make into a tea. Lotus leaf tea, lotus flower tea and lotus root tea have long been loved and still widely drunk.



#### **FOLK MEDICINE**

According to the records, hundreds of ailments are treated if it is internally used for a long time. Especially, it is believed that lotus leaf can prevent inhibit dementia.



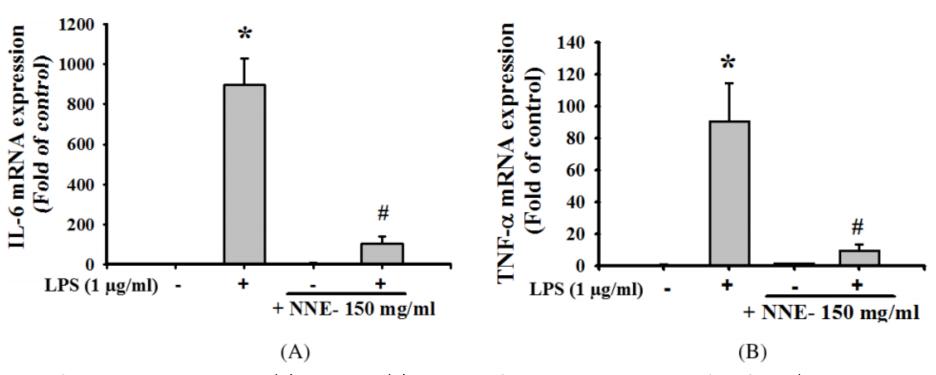
#### **FOOD**

Steamed rice wrapped in a lotus leaf is one of popular food. The leaf keeps the wrapped food fresh by preventing it from heat, humidity, and spoilage so it was used to pack food.



### Research Studies: anti-inflammatory Effect

#### Inhibition of Inflammatory Cytokines in RAW 264.7



The inflammatory cytokines, IL-6 (A) and TNF- $\alpha$  (B), were significantly inhibited in 150 mg/ml of NNE (Nelumbo nucifera Leaf Extract) treated macrophage cells.

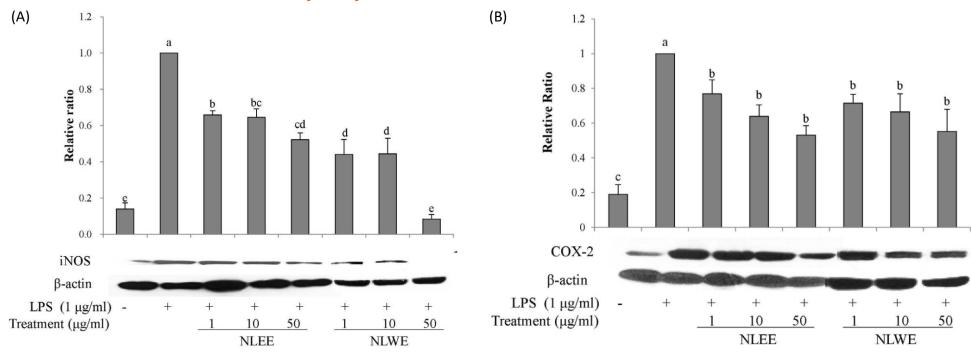
\*p < 0.05 as compared to control. #p < 0.05 as compared to LPS group.

Lotus leaf (Nelumbo nucifera) and its active constituents prevent inflammatory responses in macrophages via JNK/NF-kB signaling pathway, Liu et al., Am J Chin Med, 2014, 42(04), p.869–889



### Research Studies: anti-inflammatory Effect

#### Inhibition of Pro-inflammatory Enzymes in RAW 264.7



Effects of NLEs on iNOS (A) and COX-2 (B) expression in LPS-induced RAW 264.7 cells were identified by analyzing western blot. Cells were pre-treated with NLE for 24 h and then stimulated with LPS (1  $\mu$ g/ml).

Abbr: NLEE (Nelumbo leaf ethanol extract), NLWE (Nelumbo leaf water extract), iNOS (inducible nitric oxide synthase), COX-2 (cyclooxygenase 2)

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### in vitro Efficacy Evaluation

### \* Anti-photoaging Effect

**UVB-induced ROS Inhibition Activity** 

#### **Anti-oxidant Effect**

DPPH Radical Scavenging Activity
Superoxide Scavenging Activity

### **Skin Brightening Effect**

Melanin Synthesis Inhibition Activity

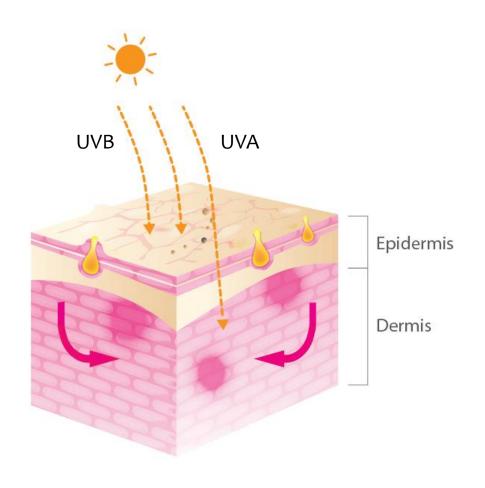
#### \* Anti-pollution Effect

Cell Recovery Activity from PM2.5-induced Damage Anti-apoptotic Activity





### What is Photoaging?



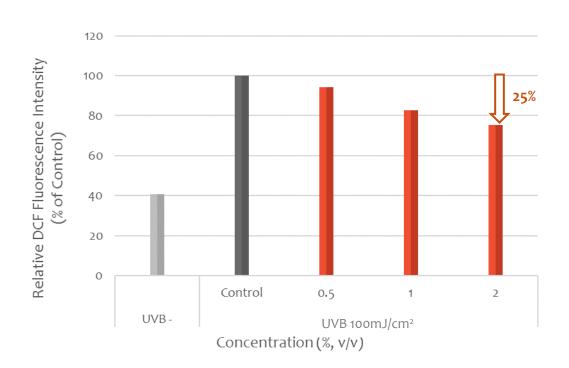
Free radicals

Photodamage
(Inflammation, wrinkle, and pigmentation)



### in vitro Efficacy Evaluation: Anti-photoaging Effect

#### **UVB-induced ROS Inhibition Activity**



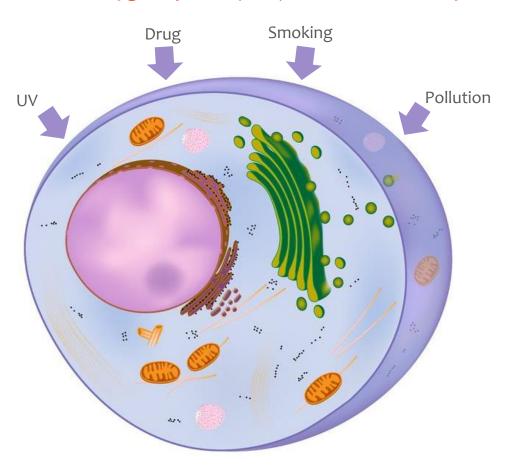
The anti-photoaging effect was measured by 2',7'-dichlorofluorescein diacetate (DCFH-DA) method.

Measurement by DCFH-DA showed that the pretreatment of Leafresh significantly reduced ROS generation caused by UVB-irradiation on human dermal fibroblasts, neonatal (HDFn) (100 mJ/cm²) in a dose dependent manner.



### **Oxidative Stress**

#### Reactive Oxygen Species (ROS) can be increased by



#### **Intracellular ROS may induce**

- DNA damage
- Lipid peroxidation
- Amino acid oxidation: protein damage
- Oxidation of co-factors: enzyme inactivation
- Chronic inflammation



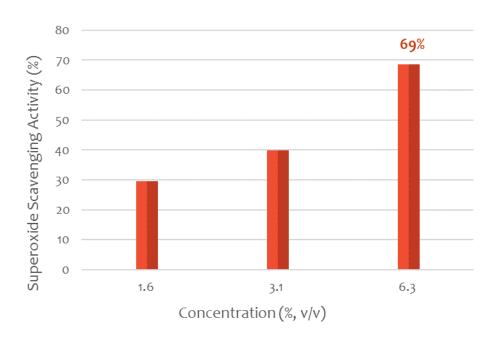


### in vitro Efficacy Evaluation: Anti-oxidant Effect

#### **\*** DPPH Radical Scavenging Activity

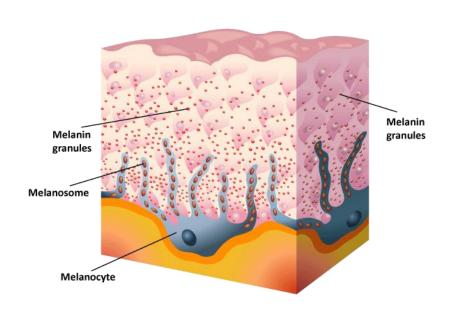
### 100 90 DPPH Scavenging Activity (%) 70 10 0.3 0.6 0.2 2.5 Concentration (%, v/v)

#### **Superoxide Anion Scavenging Activity**





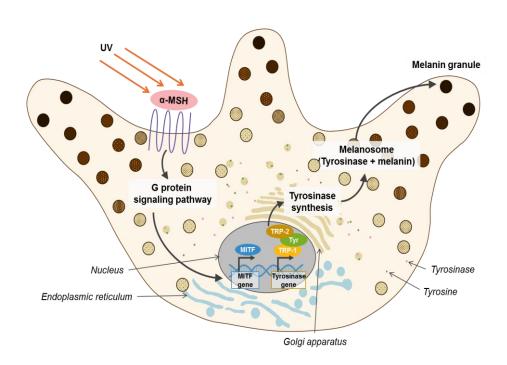
### **Skin Pigmentation**



Melanin plays an important role in protecting human skin from the harmful effects of UV sun radiation and in scavenging toxic drugs and chemicals. Melanin is synthesized in melanocytes located at the dermal/epidermal border. Epidermal melanocytes occur at an approximate ratio of 1:10 among basal keratinocytes and distribute the melanin they produce to approximately 40 overlying suprabasal keratinocytes via their elongated dendrites and cell-to-cell contacts. Skin pigmentation problems, such as melasma, freckles, age spots and dark spots, are caused by the abnormal accumulation of melanin in keratinocytes.



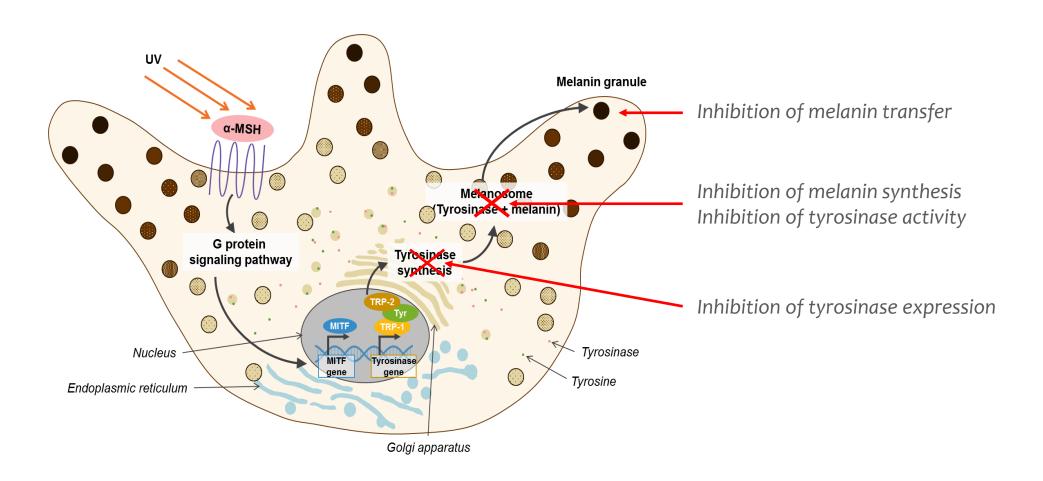
### **Melanin Synthesis**



Upon exposure to UV radiation, DNA damage triggers cytokines, growth factors and other inflammatory factors to stimulate melanin production. Melanin is synthesized in melanocytes through a series of oxidative reactions involving amino acid tyrosine in the presence of the enzyme tyrosinase. This leads to the production of melanin, and melanin granules synthesized in the melanocytes are transferred to keratinocytes.



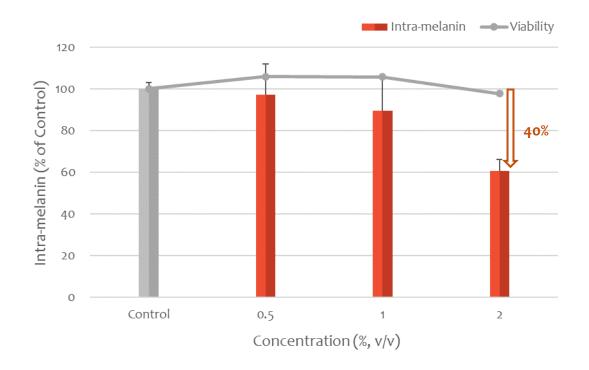
### **Skin Brightening Action**





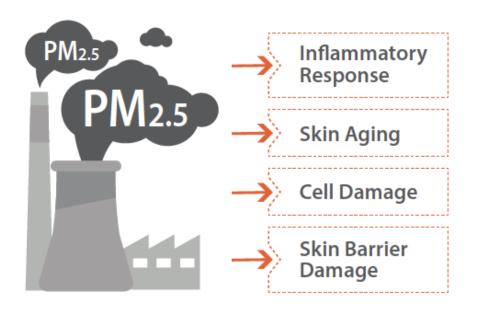
### in vitro Efficacy Evaluation: Skin Brightening Effect

**Melanin Synthesis Inhibition Activity in Melanocytes** 





### Particulate Matter (PM) Action

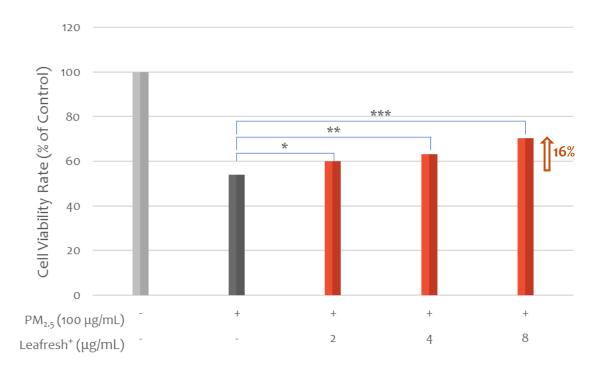


Particulate Matter (PM), known as microscopic dust, is one of the common causes of air pollution in recent years as the economy has been developed. PM includes numerous air pollutants and, among them, PM2.5 indicates particulate matter less than 2.5 µm which is secondary air pollution reacted physically and chemically with various first air pollutants. PM causes many internal damages in the human body such as inflammation, skin aging, cell damage, skin barrier damage, and so on.



### in vitro Efficacy Evaluation: Anti-pollution Effect

**Cell Proliferation Activity from PM2.5-induced Damage in HaCaT Cells** 



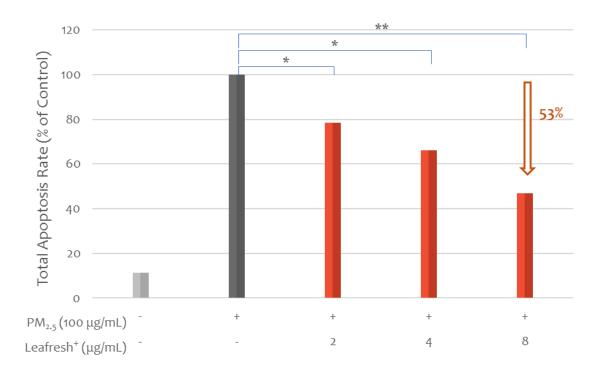
\*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 vs. Control Group (PM<sub>2.5</sub> alone)



Leafresh<sup>+</sup>: Leafresh Freeze-dried (FD) Powder Leafresh FD Powder 8 µg/mL=Leafresh(NB) 0.2%

### in vitro Efficacy Evaluation: Anti-pollution Effect

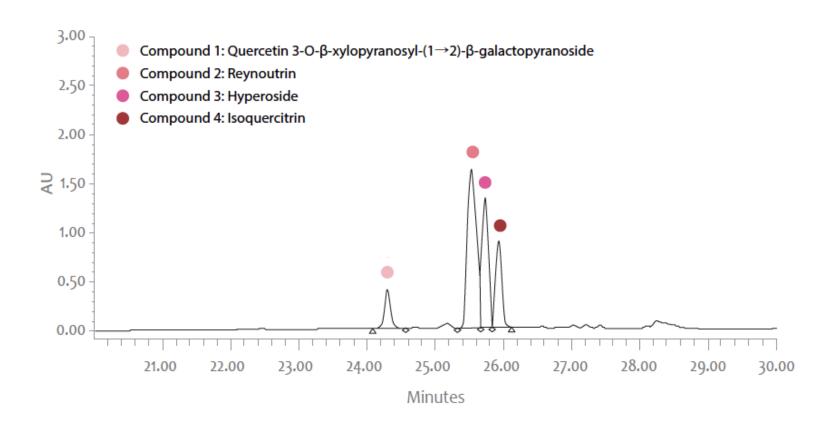
\* Anti-apoptotic Activity on PM2.5 treated HaCaT Cells





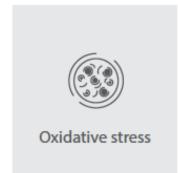


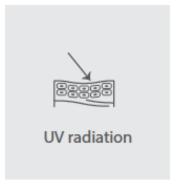
### **Active Compound Analysis: HPLC Profile of Leafresh**





### **Properties of Leafresh**









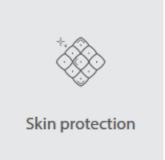














### **Marketing Points**

- Lotus leaf cultivated in Jeju Island
- Skin protection from environmental factors
- Powerful anti-oxidant activities
- Anti-photoaging effect
- **Cell protection against PM2.5**



### **Product Information**

Product Name: Leafresh(NB) COSMOS, Leafresh(PG)

Leafresh(PD)-RSPO, Leafresh(GOL)-RSPO

INCI Name: Nelumbo Nucifera Leaf Extract

**Dosage:** 1 – 3%

**Formulation:** Add to the formulation

when the temperature is lower than  $55^{\circ}$ C.

Recommended to add after the cooling process.

Storage: Avoid direct light or UV.

Keep it in a cool and dry area.





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