

GLOBAL DAILY ENVIRONMENTAL STRESS MODEL

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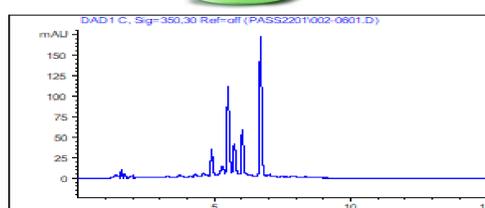
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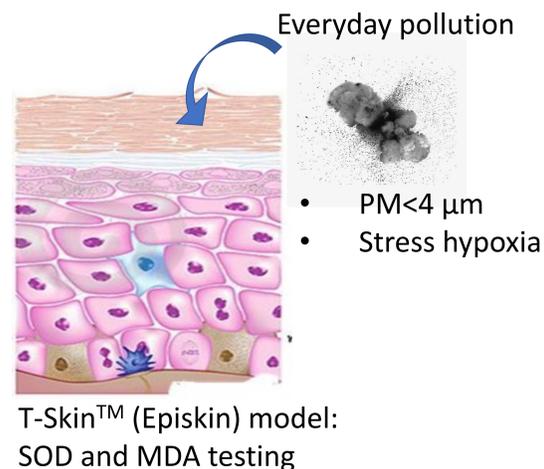


Italian alpine plants

Hydroalcoholic extraction



Chemical characterization



AIM OF THE PROJECT

An experimental model based on a new approach to the deleterious effects of everyday pollution has been developed on Full thickness skin (T-skin) reconstructed model to investigate the damages caused by a daily exposure (6h-8h) to pollutants (particulate matter, -PM < 4 microns) and hypoxic stress (Oxygen < 6%), considering that hypoxia could be associated to PM deposition on skin surface in presence of an impaired barrier function.

This model has been applied to evaluate the protection offered by different natural extracts compared to a commercial reference product with well known antioxidant action against the oxidative stress induced by environmental pollution. The oxidative stress markers considered were SOD production and MDA staining. Botanical extracts were also characterized (HPLC-DAD) in order to correlate the polyphenols content with antioxidant activity.

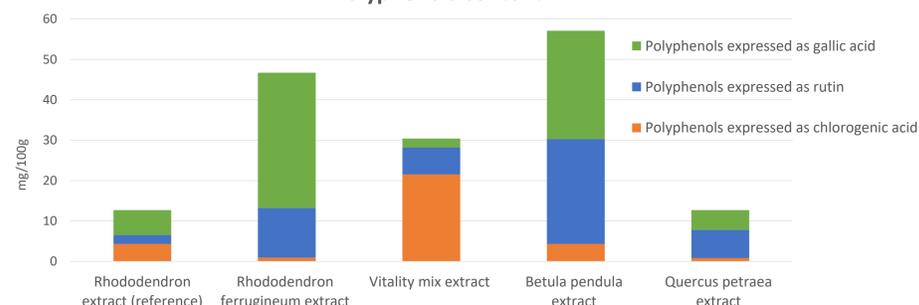
METHODS

Extraction: 50% water/ethanol mixture in an ultrasonic bath for 60 min at room temperature; filtered, concentrated using a rotary evaporator and then diluted with a mixture of glycerine and propanediol.

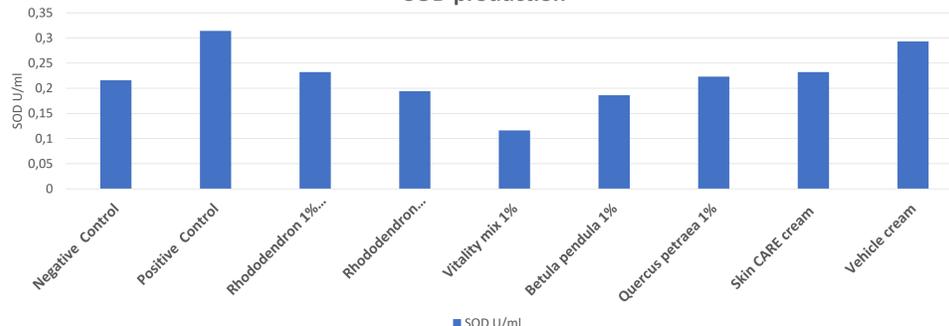
Chemical characterization: The measurements were performed with an Agilent 1260 chromatograph equipped with 1260 diode array (DAD) as detector. Separation was achieved using an Agilent Zorbax SB C-18 (4.6X150 mm) 5 μm as stationary phase. The mobile phases were water 1% formic acid and methanol. Flavonoids were quantified using rutin, chlorogenic acid and gallic acid as reference compounds.

Pollution testing: *The Superoxide Dismutase (SOD)* was measured by a Colorimetric Activity Kit (Thermo Fisher cat. EIASODC); tissues were homogenized by Precellys (Bertin) in cold PBS 1x. Optical Density (O.D.) has been read at 450 nm using the TECAN INFINITE M-200 spectrophotometer. *Malondialdehyde (MDA)* was detected by Immunohistochemistry (IHC), basing on the specificity of the antigen-binding antibody (Anti-MDA Abcam AB6463) for the detection of the target molecule marked with a chromogen of fluorophore indicator and visualized by bright field microscope (Leica DM 2500).

Polyphenols content



SOD production



The SOD assay results can be directly correlated with polyphenols content. *Rhododendron ferrugineum*, *Betula pendula* and vitality mix extracts present the highest polyphenol content and the highest response to SOD assay.

MDA Staining	MDA SIGNAL
Stress control	Increased as expected
Rhododendron extract 1% (reference)	+
Vitality mix extract 1%	+
Quercus petraea extract 1%	+
Skin CARE cream	+
Vehicle cream	=

+: positive efficacy in counteracting MDA formation; =: no significant modification compared to stress control; ND: not determined

The pre-treatment of t-Skin model with alpine extracts show a decrease of SOD production compared to positive control, indicating a prevention of ROS production.

MDA assay show positive efficacy in counteracting the MDA formation.

CONCLUSIONS

Rhododendron ferrugineum (1%), *Betula pendula* (1%) and Vitality mix (1%) have shown a good efficacy in preventing SOD enzyme expression, higher than reference commercial product. Vitality mix (1%) has shown the best anti-oxidant activity in preventing SOD enzyme expression. Skin Care Cream formulation has shown significant protective efficacy in preventing pollution damage, avoiding SOD and MDA increase.