

# Protective effect of red orange extract supplementation against UV-induced skin damages: photoaging and solar lentigines

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## Summary

**Background** Exposure of the skin to solar ultraviolet (UV) radiations causes important oxidative damages that result in clinical and histopathological changes, contributing to premature skin aging. Hyperpigmented lesions, also known as age spots, are one of the most visible alterations in skin photoaging. Skin is naturally equipped with antioxidant systems against UV-induced ROS generation; however, these antioxidant defenses are not completely efficient during exposure to sunlight. Oral antioxidants are able to counteract the harmful effects of UV radiation and to strengthen the physiological skin antioxidant defenses.

**Aims** The present study was performed to evaluate the *in vivo* skin photo-protecting and anti-aging effects of a red orange (*Citrus sinensis* varieties Moro, Tarocco and Sanguinello) extract supplementation. Previous studies showed that red orange extracts possess strong *in vitro* free radical scavenging/antioxidant activity and photo-protective effects on human skin.

**Materials/Methods** The photo-protective effects of red orange extract intake against UV-induced skin erythema and melanin production in solar lentigo was evaluated on healthy volunteers by an objective instrumental method (reflectance spectrophotometry).

**Results** Data obtained from *in vivo* studies showed that supplementation of red orange extract (100 mg/daily) for 15 days brought a significant reduction in the UV-induced skin erythema degree. Moreover, skin age spots pigmentation (melanin content) decreased from 27% to 7% when subjects were exposed to solar lamp during red orange extract supplementation.

**Conclusions** Red orange extract intake can strengthen physiological antioxidant skin defenses, protecting skin from the damaging processes involved in photo-aging and leading to an improvement in skin appearance and pigmentation.

**Keywords:** skin, red orange, oral supplementation, anti-aging, solar lentigo

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## Introduction

A localized hyperpigmented lesion, also known as age spot, solar, or senile lentigo, is one of the most visible alterations in photoaged skin, especially in Asian and Caucasian populations.<sup>1</sup> In fact, these benign-pigmented

lesions are not only considered unattractive on visible body areas (face, dorsum of the hand, upper back, and extensor forearm), but they are also a clinical marker of the degree of skin photodamage.<sup>2,3</sup> The molecular mechanisms responsible for photoaged hyperpigmentation are not completely known, but they do depend on the cumulative dose of sun exposure as well as on the amount of protection provided by its pigmentation.<sup>4</sup> Skin pigmentation is a defensive response to the production of reaction oxygen species (ROS) induced by UV radiation. Skin is naturally equipped with antioxidant systems against ROS generation; however, these antioxidant defenses are not completely efficient during exposure to sunlight, and this limitation becomes gradually more pronounced during aging.<sup>3,5</sup>

Topical application of sunscreens, which frequently contain UV filters, has been considered the first line of defense in protecting skin from UV-induced damage. Chemical sunscreens are able to absorb UV radiations, thereby protecting the skin during sun exposure. However, it has been recently recognized that their efficacy in practice is conditioned by several limiting aspects, such as inadequacy of application to the skin or removal by cutaneous perspiring.<sup>6,7</sup> Moreover, there are many concerns about the safety of UV filters due to the risk of harmful effects caused by penetration through the skin, the systemic absorption, and the UV-mediated photodegradation.<sup>7</sup>

To overcome these sunscreen limitations, the use of nutricosmetics in skin photo-protection, also known as “beauty pills”, “beauty from within” and even “oral cosmetics”, has gained considerable attention in recent years.<sup>8</sup> Among several ingredients proposed for nutricosmetics, antioxidants represent the most interesting active compounds in photo-protection due to their ability to fight free radicals generated by solar radiation.<sup>8,9</sup>

The present study was performed to evaluate the *in vivo* skin photo-protecting and anti-aging effect obtained from red orange extract intake (Citrus sinensis varieties Moro, Tarocco and Sanguinello). Previous studies showed that red orange extracts possess strong *in vitro* free radical scavenging/antioxidant activity and photo-protective effects on human skin.<sup>10–12</sup> Taken together, these reports strongly suggested that the skin protection by red orange extract is due to a block of cellular oxidative stress-related events and the inhibition of UVB-induced responses associated to inflammatory processes such as ICAM-1 (intercellular adhesion molecule-1), MCP-1 (monocytes chemoattractant protein-1), and IL-8 (interleukin-8) cellular release.<sup>10,12</sup> Moreover, the beneficial effects of blood orange extract intake on antioxidant bioavailability and on different

markers related to oxidative stress were already evaluated in healthy volunteers, such as sportsmen and smokers.<sup>13–15</sup>

In these studies, the activity of red orange fruit has mainly been attributed to the high levels of anthocyanins, (not present in blonde oranges), together with other antioxidants such as flavones, hydroxycinnamic acids, and ascorbic acid.<sup>10–15</sup> At the actual status of researches, the photoprotective properties of red orange extract cannot be attributed to a component in particular, but to the phytocomplex in its whole. The biological properties of phytocomplexes, which are constituted by several and different compounds, have been due not to one or few of its active principles, rather it is determined by a combined effects of all the components.<sup>10</sup>

The aim of this study was to investigate the *in vivo* protective effect of red orange intake in skin photo-damage and photo-aging by evaluation of UV-induced skin erythema and hyperpigmentation in sunlamp exposure test models.

## Materials and methods

### Test material

The powder extract of red orange fruit (Red Orange Complex<sup>®</sup>) used in this study was supplied by Bionap srl (Catania, Italy). It is a standardized solid extract obtained from three pigmented orange varieties (Moro, Tarocco and Sanguinello) containing the following active substances: anthocyanins (cyanidin-3-O-glucoside) 2.8–3.2% w/w, hydroxycinnamic acids (caffeic, cumaric, ferulic, sinapic acid) 1.8–2.2% w/w, flavone glycosides (narirutin, hesperidin) 8.5–9.5% w/w, and ascorbic acid 5.5–6.5% w/w. Capsules filled with red orange extract were prepared for the *in vivo* studies with a daily dosage of 100 mg of extract for each subject.

### Subjects

*In vivo* experiments were performed on healthy volunteers recruited after medical screening including the completion of a health questionnaire followed by physical examination of the application sites. Subjects exhibiting such features as abnormal sensitivity to sunlight, obesity, active smoking, occupational exposure to toxic agents, the use of antioxidant dietary supplementation, and vegetarian dietary habits were excluded from the study. After they were fully informed of the nature of the study, substances and procedures involved, they gave their written consent. No subjects were taking medications (including vitamin and antioxidant supplements)

and were required not to modify their dietary habits during the course of the study. Moreover, to minimize any environmental influence on daily doses of UV radiation exposure, studies were started in November, and each subject was advised to avoid exposure to the sun and use of sunscreen on the treated skin.

#### *In vivo* evaluation of UV-induced skin erythema

The *in vivo* evaluation of skin erythema induced by UV irradiation was performed on twenty Caucasian subjects of skin types II and III (aged 26–47 years). For each subject, two sites on the ventral surface of each forearm were defined using a circular template (1 cm<sup>2</sup>) and demarcated with permanent ink. Afterward, in each test site, skin erythema was induced by UV-B irradiation using an ultraviolet lamp, model UVM-57 (UVP, San Gabriel, CA, USA) which emitted in the range 290–320 nm with an output peak at 302 nm. The flux rate measured at the skin surface was 0.80 mW/cm<sup>2</sup> and the irradiation dose corresponded to double of the minimal erythema dose (MED) of each subject. The erythema induced by lamp exposure was monitored on the skin sites at different time points for 48 h (2, 8, 12, 25, 35, and 48 h) using a reflectance visible spectrophotometer X-Rite model 968 (X Rite Inc. Grandville, MI, USA), having 0° illumination and 45° viewing angle.<sup>16</sup> Reflectance spectra were obtained over the wavelength range 400–700 nm using illuminant C and 2° standard observer. After a rest period of 3 weeks, the same subjects were treated with 100 mg/die of red orange extract for a period of 15 days. At the end of red orange extract supplementation, skin sites on the forearms of subjects were exposed to UV irradiation, and the induced skin erythema was monitored by reflectance spectrophotometry for 48 h, as previously reported.

From the spectral data, skin erythema index (E.I.) values were calculated by subtracting the logarithm of inverse reflectance (log 1/R) values at 510 and 610 nm (mainly due to melanin absorption) from the sum of log 1/R values at 540, 560, and 580 nm, which represent the wavelengths of hemoglobin absorption peaks (Eqn. 1).<sup>16</sup>

$$\text{E.I.} = 100 \left[ \log \frac{1}{R_{560}} + 1,5 \left( \log \frac{1}{R_{540}} + \log \frac{1}{R_{580}} \right) - 2 \left( \log \frac{1}{R_{510}} + \log \frac{1}{R_{610}} \right) \right] \quad (1)$$

Afterward, variation of the erythema index ( $\Delta$ E.I.) for each skin sites was calculated by subtracting E.I. baseline values from the E.I. values obtained at

different time points during the monitoring period of the study. For each site, plotting  $\Delta$ E.I. vs. time, the area under the curve was computed using the trapezoidal rule to obtain AUC (area under curve) dimensionless index values directly related to the degree of skin erythema. All the regions were measured in triplicate.

#### *In vivo* evaluation of skin appearance homogeneity during sunlamp exposure

The *in vivo* study was carried out for a period of 5 weeks on 25 volunteers of skin types II and IV, aged 45–70 years (mean of 56 years), recruited after dermatological screening and all having at least five solar lentigo (spots) on the dorsa of each hand. For each subject, three solar spots (mean size 8–10 mm) were selected, and one spotless area was demarcated with permanent ink using a circular template (1 cm<sup>2</sup>) and considered as a control on each hand. Experimental design of the study was reported in Table 1. In details, in the 1st week of the study, solar spots (A) and spotless sites (B) located on one hand for each subjects were exposed to tanning treatment by a lamp simulating sunlight (Helios Italquartz srl, Milan, Italia), which emitted the range of 300–400 nm (6.5 mW/cm<sup>2</sup>), for 2–8 min depending on the minimal erythema dose (MED). To avoid induced and interfering skin erythema events, skin exposure to the lamp was not conducted on the third and sixth day of the week. The induced pigmentation on spots (A) and spotless sites (B) of the hand was monitored at the end of the 1st week and the 2nd week, when skin reached highest pigmentation, as observed in previous research.<sup>16</sup> After a rest period of 1 week (3rd week), spots (C) and spotless sites (D) located on the other hand of the same subjects

**Table 1** Experimental design of the *in vivo* evaluation of skin tanning induced by sunlight lamp exposure of solar spots (A and C) and spotless skin sites (B and D) located on the hands (dorsa) for each subjects

No supplementation	
1st week	Tanning treatment by sunlight lamp exposure of spots (A) and spotless sites (B) located on the same hand and evaluation of the induced pigmentation
2nd week	Evaluation of skin melanin development of spots (A) and spotless sites (B)
3rd week	Rest period
Oral supplementation with red orange extract (100 mg/die)	
4th week	Tanning treatment by sunlight lamp exposure of spots (C) and spotless sites (D) located on the other hand and evaluation of the induced pigmentation
5th week	Evaluation of skin melanin development of spots (C) and spotless sites (D)

were exposed to sunlight lamp treatment on the 4th week of the experimental study, and the induced pigmentation of skin sites was monitored at the end of the 4th and the 5th week, as previously reported, during red orange extract supplementation (100 mg/die, for 15 days). From the reflectance spectral data, the melanin index (M.I.) of spots (A,C) and spotless sites (B, D) was obtained using the following equation (Eqn. 2):<sup>16</sup>

$$\text{M.I.} = \left( \log \frac{1}{R_{650}} - \log \frac{1}{R_{700}} \right) + 0.015 \quad (2)$$

where the log of inverse reflectance values ( $\log 1/R$ ) is the apparent absorbance at a specific wavelength (650 and 700 nm), and 0.015 is an adjusted instrumental factor. This index is calculated as the slope of the apparent absorbance levels from 650 to 700 nm and was used to measure both melanin and melanogenic dose–response. Thereafter, the mean  $\Delta$ M.I. values (variation of melanin index vs. baseline) obtained from the solar spots were compared to  $\Delta$ M.I. values of spotless skin sites contained on the same hand (A vs. B and C vs. D), and the homogeneity of skin pigmentation was expressed as tanning variation percentage (TV%), using the following equation (Eqn. 3):

$$\text{TV}(\%) = \frac{\Delta\text{M.I.}_s - \Delta\text{M.I.}_c}{\Delta\text{M.I.}_c} \times 100 \quad (3)$$

where  $\Delta$ M.I.<sub>s</sub> is the variation in melanin index induced by sunlamp exposure for the solar spots (A,C), and  $\Delta$ M.I.<sub>c</sub> is the variation in melanin index of spotless sites (B,D). All the regions were measured in triplicate.

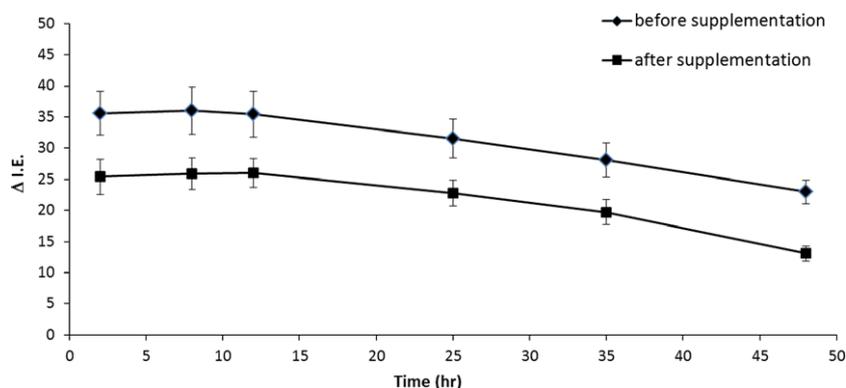
#### Statistical analysis

All data obtained were submitted to a statistical analysis. All statistical comparisons in instrumental

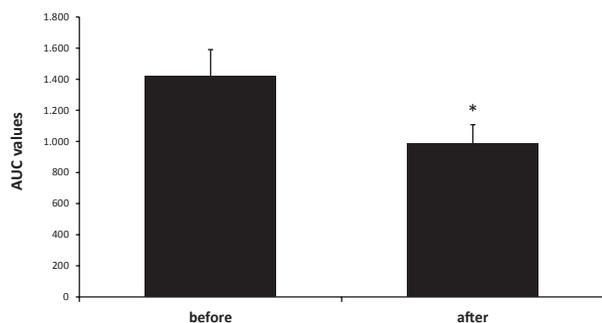
assessment were evaluated using repeat-measure analysis of variance (ANOVA) followed by the Bonferroni–Dunn *post hoc* pair-wise comparison procedure. A *P* value of < 0.05 was considered significant.

## Results

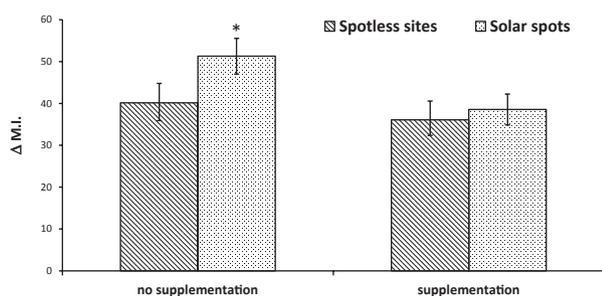
The *in vivo* evaluation of skin erythema induced by UV irradiation showed that supplementation of red orange extract (100 mg/die) for 15 days brought about a significant reduction in the skin erythema degree in subjects. The trends in mean E.I. variation ( $\Delta$ E.I.) vs. time (48 h) before and after supplementation for subjects are reported in Fig. 1. Results showed a mean reduction of approximately 40% of the UV-induced skin erythema after oral supplementation, as evidenced by AUC values reported in Fig. 2. From the results of the *in vivo* evaluation of tanning skin homogeneity, it was observed that a very low increase in melanin content in spot (A, C) and spotless sites (B, D) was obtained at the end of the sunlight lamp exposure (data not shown). As expected, skin pigmentation occurred the week following sunlight lamp exposure, and a significant increase in melanin content was observed in the skin.<sup>16</sup> At this time point,  $\Delta$ M.I. value obtained from the solar spots A and C was calculated and compared to the values obtained from the spotless site B and D, respectively, for each subject. From the data obtained (Fig. 3), it was observed that  $\Delta$ M.I. values of solar spots A were significantly higher ( $P < 0.05$ ) than  $\Delta$ M.I. values of spotless sites B, whereas the increase in melanin content of solar spots C was not significantly higher than spotless skin sites D for the same subjects ( $P < 0.05$ ). In fact, it was observed that the mean value of TV% decreased from 27% to 7% when the same subjects were exposed to sunlight lamp treatment during red orange extract supplementation.



**Figure 1** Trend of UV-induced skin erythema index ( $\Delta$ E.I.) vs. time (48 h) from subjects before and after red orange extract supplementation.



**Figure 2** Mean area under curve values (AUC  $\pm$  DS) of UV-induced skin erythema from subjects before and after red orange extract supplementation; \* $P < 0.05$  (significantly different).



**Figure 3** Mean values of  $\Delta$ M.I. (variation of the melanin index vs. baseline) of solar spots and spotless skin sites from one hand treated before (A and B) and from the other hand of the same subjects during red orange extract supplementation (C and D), \* $P < 0.05$  significant different vs. spotless sites.

## Discussion

In recent years, skin care science has emphasized the importance of food phytochemical supplements in combating skin aging and disorders. The well-known relationship between nutrition and human health, including skin condition, has recently led to the modern concept of “skin care from within”.<sup>8</sup> In particular, skin protection against damage induced by sun exposure has gained a great deal of interest.<sup>1</sup> In fact, the use of nutricosmetics or “beauty pills” in skin photoprotection seems to be particularly appealing as their efficacy is not dependent on the adequacy of topical application.<sup>9</sup> Many studies have already shown that some health nutrients, such as vitamin A, E and C, and herbal extracts, protect skin because of their antioxidant activities.<sup>8,17,18</sup> Oral antioxidants are able to counteract the damage processes induced by UV radiation and to strengthen the physiological skin antioxidant defenses.<sup>9</sup>

In this study, the *in vivo* protective effects of red orange intake against UV-induced skin damage and skin photo-aging were evaluated. Red oranges (Moro, Tarocco and Saguinello varieties) are characterized by high content of antioxidant natural compounds such as flavonoids (anthocyanin, flavones, hydroxycinnamic acids) and ascorbic acid.<sup>19</sup> Red orange intake has been shown to have several biological applications, and the antioxidant effect is already well known in literature.<sup>10–15</sup>

Previous studies showed that red orange extract possesses strong *in vitro* free radical scavenging/antioxidant activity and photo-protective effect against UVB-induced skin responses.<sup>10–12</sup> Cimino *et al.*<sup>10</sup> strongly suggested that the protection by red orange extract is exerted through an antioxidant mechanism, either by direct shielding of UV radiation or by improving the antioxidant cellular network. Researchers indicated that it is potentially able to efficiently counteract UVB-induced response, by blocking cellular oxidative stress-related events, in cultured human keratinocytes. In particular, events related to inflammation and apoptosis, such as NF- $\kappa$ B and AP-1 translocation and procaspase-3 cleavage, are significantly affected by red orange extract pretreatment.<sup>10</sup> Moreover, Cardile *et al.*<sup>12</sup> have proven that red orange extract markedly decreased expression of membrane molecules (ICAM-1) and the release of inflammatory soluble factors (MCP-1 and IL-8) induced by pro-inflammatory mediators in normal human keratinocytes cell line (NCTC 2544).<sup>12</sup> Finally, *in vivo* bioavailability of the antioxidant effect of red orange extract supplementation was already evaluated.<sup>13–15</sup> Dietary supplementation with red orange extract was able to counteract human oxidative stress status by increasing serum thiol groups in healthy volunteers who smoke and in sportsmen.<sup>13–15</sup>

In this study, it was observed that supplementation with red orange extract containing antioxidant compounds of these fruits is able to protect skin against sun exposure damage. The *in vivo* test models have proven that red orange extract can inhibit skin erythema processes induced by UV radiation. Moreover, red orange intake can counteract skin hyperpigmentation of solar lentigo and improve the appearance of skin and the homogeneity of skin tanning through the inhibition of melanin overproduction caused by repeated UV exposure. To this purpose, the increase of melanin content in solar lentigenes (sun spots) has been monitored by objective instrumental method (reflectance spectrophotometry) in healthy volunteers before and after oral supplementation, while undergoing sunlamp exposure. Results showed that UV exposure

can increase pigmentation of spot sites, but a significant variation of melanin content between spot and spotless skin sites, used as control, was observed before supplementation with red orange extract. As consequence, higher skin color homogeneity was obtained by oral supplementation during sunlamp exposure. The *in vivo* effects of the extract intake observed in these studies may be correlated to the antioxidant actions of active compounds contained in orange varieties and their ability to counteract the sensibility of hyperpigmented spots to UV exposure.

It is already well known that when UV radiation penetrates into the human skin, several defensive molecular events occur that result in clinical and histopathological changes (erythema, edema, hyperpigmentation, and rough texture) contributing to premature skin aging.<sup>1,17</sup> Even if molecular mechanisms are still not completely understood, UV-induced oxidative stress and ROS generation within the skin seems to play a major role in these cutaneous changes. In particular, ROS species can act on several skin processes such as UV-induced melanogenesis and inflammation.<sup>5</sup> ROS can directly stimulate melanocytes to produce excess melanin through different molecular pathways.<sup>4</sup> The increase in NO production and DAG (diacylglycerol) intracellular content seems to be mainly correlated to direct melanogenic effects of UV on melanocytes. Moreover, among ROS, NO radical species derived from adjacent keratinocytes can also induce melanogenesis by increasing the amount of the melanogenic factors tyrosinase and tyrosinase-related protein 1.<sup>5</sup> Oxidative stress and ROS production in the skin seem to be strictly correlated to UV-induced inflammatory processes too.<sup>4,5</sup> It was observed that ROS can stimulate transcription nuclear factor such as NF- $\kappa$ B, leading to an increase in pro-inflammatory cytokines release. In turn, pro-inflammatory cytokines can stimulate the epidermal keratinocytes and dermal fibroblasts, upregulate metalloproteases levels, and degrade dermal collagen and elastic fibers.<sup>9,17</sup> Therefore, skin inflammation induced by acute or long-term exposure to sunlight (sunburn) can strongly contribute to the acceleration of skin-aging processes. Furthermore, earlier studies have shown that a correlation between skin inflammatory processes and melanogenesis could also occur, and low-level chronic inflammation can stimulate the development of hyperpigmented solar lentigenes in the skin.<sup>1</sup>

On the basis of these assumptions, it is possible to hypothesize that red orange extract intake can strengthen physiological antioxidant skin defenses, protecting skin from the damaging processes involved in photo-aging and leading to an improvement in skin

appearance and pigmentation. In conclusion, the natural blend of red orange active compounds (varieties Moro, Tarocco, and Sanguinello) can be considered as a good candidate as a nutraceutical ingredient in skin photo-protection and skin care.

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