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Emblica Cascading Antioxidant: A Novel Natural Skin Care Ingredient

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Key Words

Antioxidant · Pro-oxidant · Transition metals · Oxidative stress · Skin care · Natural product · Hydrolyzable tannins · *Phyllanthus emblica*

Abstract

A standardized extract of *Phyllanthus emblica* (trade named Emblica) was found to have a long-lasting and broad-spectrum antioxidant activity. The product has no pro-oxidation activity induced by iron and/or copper because of its iron and copper chelating ability. Emblica helps protect the skin from the damaging effects of free radicals, non-radicals and transition metal-induced oxidative stress. Emblica is suitable for use in anti-aging, sunscreen and general purpose skin care products.

Emblica (a trade name product of Merck KGaA, Darmstadt, Germany and EM Industries, Inc., USA, an affiliate of Merck KGaA) cascading antioxidant is a well-defined material, isolated from the fruits of *Phyllanthus emblica* (syn. *Emblica officinalis* Gaertn). This plant ranges in status from insignificant in the Western world to highly prized in tropical Asia. The tree is graceful ornamental, normally reaching a height of 60 ft (18 m) and in rare instances of 100 ft (30 m). *P. emblica* is one of the important Ayurvedic ('science of life') herbs in India, and has been used for over thousands of years for a wide variety of human ailments [Chopra et al., 1956]. Our study on the antioxidant constituents of *P. emblica* is centered around its skin care benefits, as there has been no systematic work done so far on this product. Product attributes of Emblica are summarized below:

Product Attributes

- A safe and effective natural antioxidant
  Well-defined material
  Pro-oxidation-free antioxidant
  Dual functionality: chelation and antioxidant, two functions are separated
- Cascading effect provides long-lasting activity

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- Statistically significant reduction in UV-induced pigmentation (results not included)
- Protection of skin fibroblast cells against oxidative stress (in vitro)
- Excellent safety profile – edible fruit.

Product Standardization

The fruits are selected, harvested and processed according to strict criteria to ensure a consistently high-quality product. The quality criteria are based on extensive research on chemotypes, geographical location of the plants, time of harvesting and on the extraction process to obtain consistently good-quality material. Emblica cascading antioxidant is extracted from premium quality fruits using a water-based process [US Patent No. 6,124,268]. Emblica cascading antioxidant is distinctly different from other commercially available extracts of *P. emblica* fruits, as it is defined to the extent of well over 50% in terms of its key chemical components. None of the extracts of *P. emblica* in the market compares to Emblica cascading antioxidant in composition and consistency of composition, and color.

The low-molecular-weight hydrolyzable tannins (<1,000), namely Emblicanin A and Emblicanin B, along with Pedunculagin and Punigluconin are the key ingredients in Emblica cascading antioxidant. The literature continues to claim the presence of stable vitamin C in *P. emblica* as the key ingredient, which has been proven to be erroneous [Ghosal et al., 1996]. In nature, Emblicanin A and Emblicanin B have only been found in *P. emblica* plants [Ghosal et al., 1996]. The structures of the monomeric compounds are given in figure 1.

Emblica cascading antioxidant has been standardized [Monograph, 2001] by using high-performance thin-layer chromatography with silica gel TLC plate, E. Merck, and a solvent system consisting of ethyl acetate/formic acid/acetic acid/water. Alternately, the product can be standardized by using high-performance liquid chromatography.

Pro-oxidation-Free Antioxidant

To control oxidative processes, i.e. to reduce, if not prevent, their harmful effects on the skin, diverse antioxidants can be used to protect the skin from photodamage. When a general use of antioxidants is advocated, it is often disregarded that these compounds not only function as antioxidants, but (intrinsically) have a pro-oxidant action [Bast et al., 1991], especially in the presence of transition metals like iron and copper. Release of iron from the iron-storage protein ferritin under UV light has been ascribed to be the main source of oxidative stress [Brenneisen et al., 1998; Pourzand et al., 1999]. The consequent release of potentially harmful free iron within the cells will clearly exacerbate the damaging effects of photoperoxidation and is likely to be of central importance to both reversible and degenerative damage to the skin after exposure to UV light. It has been shown that the iron content of human epidermis is three-fold greater in sun-exposed areas than in nonexposed body sites [Bissett and McBride, 1992]. Iron exerts its toxicity through a series of reactions with reactive oxygen species called the Fenton reaction, generating the highly toxic hydroxyl radical with subsequent damage to biomolecules [Halliwell and Gutteridge, 1998]. Emblica cascading antioxidant is completely free of pro-oxidation activity induced by transition metals, whereas well-known antioxidants like vitamin C, vitamin E, proanthocyanidins (from pine and grape), superoxide dismutase and glutathione do have prooxidative activity [Bast et al., 1991]. Chemis-

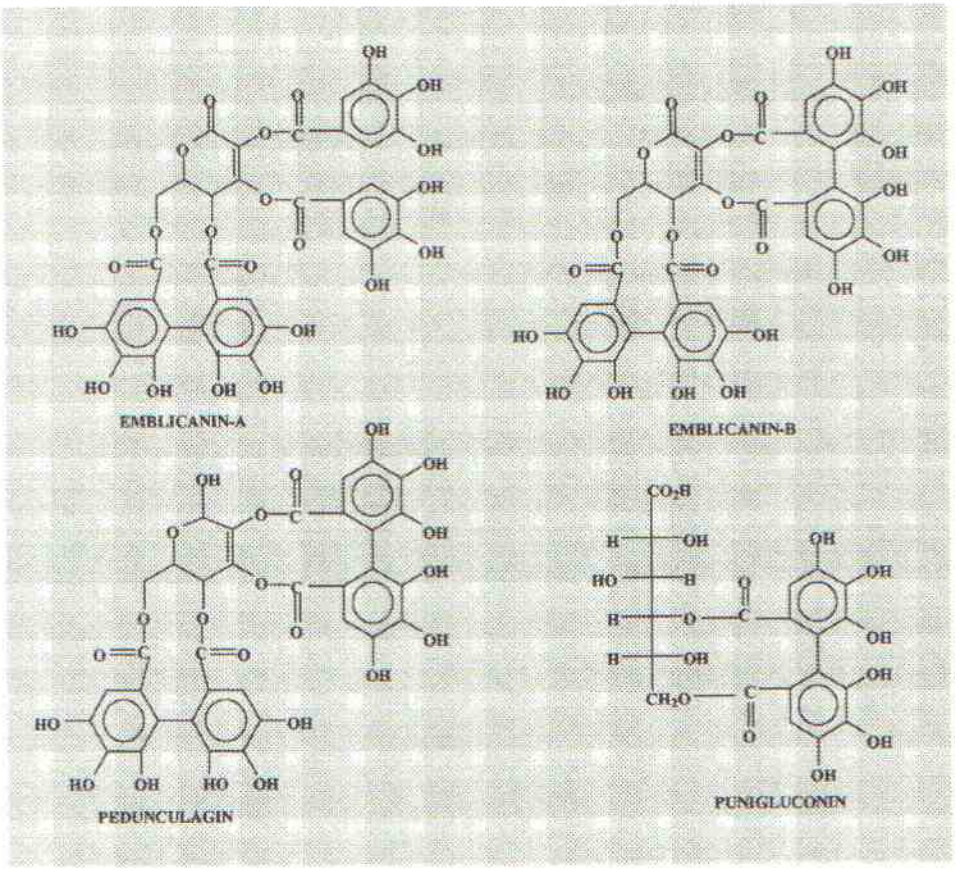

Fig. 1. Structures of key ingredients in Emblica cascading antioxidant.

try involved in transition metal-induced pro-oxidation is summarized below:

Presence of iron and H_2O_2:

$Fe^{2+} + H_2O_2 \rightarrow$ intermediate complex(es) $\rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$ (very fast reaction)

$Fe^{3+} + H_2O_2 \rightarrow$ intermediate complex(es) $\rightarrow Fe^{2+} + O_2^{\cdot-} + 2H^{+}$ (slow reaction)

Presence of Fe^{3+} chelates and H_2O_2:

Fe^{3+}-EDTA + $H_2O_2 \rightarrow Fe^{2+}$-EDTA + $O_2^{\cdot-} + 2H^{+}$

Presence of iron (or copper), H_2O_2 and an antioxidant:

Fe^{3+} + ascorbate $\rightarrow Fe^{2+}$ + ascorbate$^{\cdot}$

Fe^{2+} + $H_2O_2 \rightarrow$ [intermediate complex(es)] $\rightarrow Fe^{3+} + HO^{\cdot} + HO^{-}$

Sunlight also generates superoxide on the skin and increases the presence of superoxide dismutase, which converts the superoxide to hydrogen peroxide. Fe^{2+} reacts with the hydrogen peroxide to generate the destructive

Table 1. UV spectral data of Fe^{3+} chelators

Chelator/antioxidant	Absorption maxima of complex, λ_{max} nm	
	with Fe^{3+}	N_3 induced shift
EDTA	241, 283	241, 283, 410
Emblica	**241, 294, 353, 377**	**241, 294, 353, 377/no shift**
Pine antioxidant	241, 294, 353, 384	241, 294, 353, 400, 440
Vitamin C	238, 262	241, 266, 295
Grape antioxidant	247, 295, 353, 396	247, 295, 353, 415, 430
Green tea antioxidant	240, 272, 324, 390	240, 277, 325, 390
Gallic acid	247, 295, 337	247, 295, 353, 412

The peak positions are obtained from differential spectroscopic scans of 1.0 m*M* Fe^{3+} and 5 m*M* chelator, 1.0 *M* NaN_3, 50 m*M* phosphate buffer, pH 7.4, versus the same solution without sodium azide. Underlining of figures indicates new peak(s) or peak(s) have been shifted.

hydroxyl radical and Fe^{3+}, which allows the chain reaction to continue. In the presence of antioxidants/pro-oxidants like vitamin C, vitamin E or other polyphenolics, one has a perfect oxidative chemistry ready to take place on the skin.

Emblica cascading antioxidant is an excellent chelator for Fe^{3+} and Cu^{2+}, thereby eliminating the generation of the hydroxyl radical and its detrimental effects on the skin. As an antioxidant, it quenches free radicals that happen to form on the skin. Iron-catalyzed formation of hydroxyl radical from superoxide anion radical and hydrogen peroxide requires the availability of at least one iron coordination site that is either empty or occupied by a readily dissociable ligand such as water. This coordination with water may be completely displaced by stronger ligands like azide (N_3^-) anion. We have applied this principle and determined if any coordination site is free in the Fe^{3+}-antioxidant complex by the UV spectrophotometric method [Graf et al., 1984; Martell et al., 1957]. The results are shown in table 1.

Cascading Effects

While most antioxidants go from an active to an inactive form, Emblica cascading antioxidant utilizes a multilevel cascade of antioxidant compounds (shown below), resulting in a long-lasting and stable antioxidant activity. This cascading effect was proven by tests of inhibitory activity of Emblica constituents and their metabolites on lipid peroxidation [Halliwell and Gutteridge, 1998; Ohkawa et al., 1979].

Emblicanin A → Emblicanin B → Emblicanin oligomers.

Stability in Aqueous and Emulsion Systems

The diphenylpicrylhydrazide (DPPH) method [Kato et al., 1988] was used to determine the long-lasting antioxidant activity of Emblica cascading antioxidant. The result is summarized in figure 2.

The DPPH method is a simple colorimetric assay of determining antioxidant activity based on the decrease in absorbance at

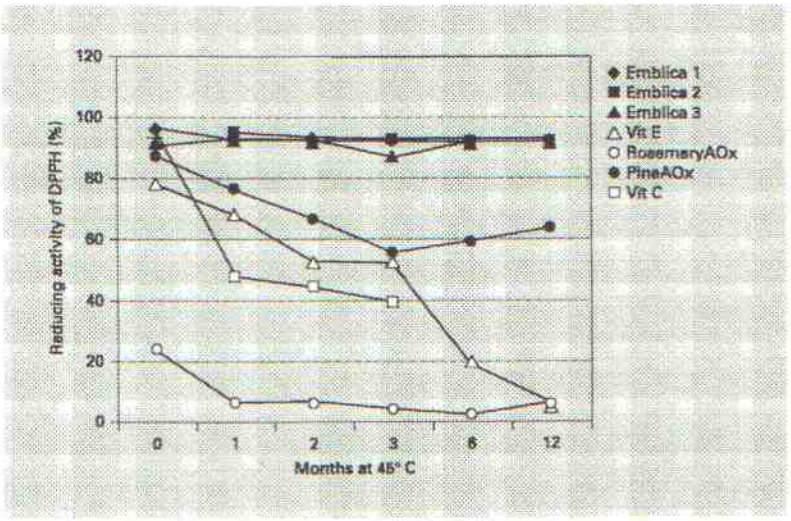

Fig. 2. Comparative antioxidant activity of Emblica cascading antioxidant and other natural antioxidants. All antioxidant activities were derived from optical density measurements at 517 nm in ethanol-water with concentrations as follows: DPPH at 100 μM/ml; all antioxidants at 31 µg/ml except rosemary antioxidant at 61 µg/ml. Vit = Vitamin; RosemaryAOx = rosemary antioxidant; PineAOx = pine antioxidant.

517 nm of the DPPH radical (deep purple) after the addition of an antioxidant compound in an aqueous ethanolic solution. Emblica cascading antioxidant retains antioxidant activity to the fullest extent after 12 months at 45 °C, while others failed. Additionally, a minimal batch-to-batch variation in antioxidant activity of Emblica cascading antioxidant is seen in this test.

Protection of Skin Fibroblast Cells under Oxidative Stress (In vitro)

The direct biological damage that can be caused by superoxide is highly selective and often involves its reaction with other radicals. Thus, superoxide can reduce Fe^{3+}, but also oxidize Fe^{2+}. We have used the hypoxanthine-xanthine oxidase system [Richard et al., 1992] to generate superoxide to determine protection of human dermal fibroblast cells using Emblica cascading antioxidant. Cell viability under oxidative stress was determined by measuring optical density at 570 nm. The results are summarized in table 2. Emblica provides about 87% protection of human dermal fibroblast cells under oxidative stress.

Safety Profile

Acute Oral Toxicity Study in Rats – Limit Test

An acute oral toxicity test was conducted with rats to determine the potential for Emblica to produce toxicity from a single dose via the oral route. Under the conditions of this

Table 2. Protective effect of Emblica csascading antioxidant on human skin fibroblast cells under oxidative stress

	Control	HX-OX	Emblica 1	Emblica 2
Optical density at 570 nm	0.755	0.22	0.68	0.63
Protection of cells against oxidative stress	–	–	90%	83%

HX-OX = Hypoxanthine-xanthine oxidase.

study, the single-dose acute oral LD_{50} is >5,000 mg/kg of the body weight in male and female rats.

Primary Eye Irritation Study in Rabbits

A primary eye irritation test was conducted with rabbits to determine the potential for Emblica to produce irritation from a single instillation via the ocular route. Under the conditions of this study, the test substance is classified as minimally irritating to both the unrinsed and rinsed eyes.

Evaluation of Phototoxicity Potential by UVA Irradiation on Human Subjects

A phototoxicity test was conducted with 20 human subjects to determine the potential for Emblica to produce phototoxicity from a single dermal application. Under the conditions of this study, the test substance is classified as nonphototoxic when tested on human subjects at 2% dilution in distilled water.

Repeat Insult Patch Test on Human Subjects/Skin Irritation and Skin Sensitization Evaluation

A repeat insult patch test was conducted with 100 human subjects to determine the potential for Emblica to produce primary irritation and primary sensitization. Under the conditions of the study, the test substance is considered as a nonprimary irritant and a nonprimary sensitizer to the human skin.

Salmonella Mutagenicity Test

A salmonella mutagenicity test was conducted to determine the potential for Emblica at 50 and 100 μg/plate to produce mutagenicity. Under the conditions of the study, the test substance is considered as a nonmutagenic material.

Conclusion

Emblica cascading antioxidant has an excellent toxicity profile and has no adverse biological effects on humans. Thus, Emblica cascading antioxidant is a safe, suitable and effective product for use in antiaging, sunscreen and general-purpose skin care and skin protection products as well as in color cosmetics. Emblica cascading antioxidant, having no pro-oxidation property, may provide a greater value than the existing antioxidants as a stand-alone or in combination with sunscreens for skin care products of the future.

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